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El caso Schizopora Xylodon: integrando diferentes metodologías en el estudio de la diversidad fúngica

The Schizopora Xylodon case: integrating different methodologies in the study of fungal diversity

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Summary

Introduction

Fungi are considered key organisms across all ecosystems, mainly because of the fundamental roles they play in essential ecological processes from nutrient recycling to symbiotic associations. Fungi are also among the most numerous organisms. Despite these prominent roles, their diversity is largely unnamed, since it is estimated that only 3% to 8% of species have been described to date. Traditionally, the study of fungal species delimitation has been based upon morphological characters. However, with the development of new techniques, DNA sequences have provided more convincing evidence for species delimitation and classification. This is because changes in genetic sequences occur frequently and can be detected more easily than subtle changes in other traits, such as morphological characters, and therefore DNA sequences can reveal hidden species masked under a single species name. Despite the success of DNA sequences for documenting biodiversity, especially the ITS region of the nuclear ribosomal DNA (the barcode for fungi), taxonomists and evolutionary biologists have come to recognize the importance of including multiple and complementary sources of evidence for species delimitation in an integrative taxonomy framework.

Schizopora (Basidiomycota, Hymenochaeteales) is a genus of corticioid fungi that include species of wood decomposers distributed worldwide. It is characterized by a hymenophore with pores that break apart to form teeth, among other traits. However, as a result of recent molecular studies *Schizopora* species were transferred into the genus *Xylodon*. *Xylodon* is considered one of the most species rich corticioid genera with more than 80 species formally recognized. During the last ten years, 14 new species have been described and 59 combinations have been proposed, which shows that *Xylodon* taxonomy is far from being completely known. This taxonomic instability in combination with the supposed worldwide distribution for many of the species makes *Xylodon* a good candidate for using an integrated taxonomy framework to study its species diversity.

Objectives and results

Our working hypothesis is that the characters and approaches used in the study of fungal diversity affects to the results obtained in terms of number, distribution and diagnostic

traits of species. To test this hypothesis, morphological, molecular and ecological analyses are conducted over a group of selected species in *Xylodon*. Particularly, we address the following objectives: A) to evaluate how the sources of taxonomic evidence affect the detectable number of candidate species and their distribution patterns; B) to look for new sources of taxonomic evidence that allow better recognition of diverse species; and C) to investigate the available methodologies to combine different sources of evidence for the delimitation of species.

This dissertation is structured in five chapters, which address the objectives previously described, and has obtained the following results:

In Chapter 1 the effect of the taxonomic uncertainty is explored in distribution models of three *Xylodon* species. Worldwide distributions were obtained when names in herbarium and sequence vouchers were used for species identification. However, when the same specimens were subjected to a molecular study together with the GenBank sequences, up to 19 species candidates were obtained with restricted or regional distributions.

In Chapter 2 the study of the *Xylodon* raduloides complex is addressed using three different sources of taxonomic evidence: molecular, morphological and ecological. This study unmasked four different species under a single name, with a strong biogeographic pattern: *Xylodon laurentianus* sp. nov. in North America, *X. novozelandicus* sp. nov. in Australia-New Zealand, *X. patagonicus* sp. nov. in the Patagonian region, and *X. raduloides* in Europe.

In Chapter 3 the taxonomy of the *Xylodon australis* complex is analyzed using molecular and morphological data. In addition, a maximum likelihood based approach is used to integrate both sources of evidence. Thanks to this study, two different species can be distinguished under the *Xylodon australis* name, and the kinship relationships were clarified. Moreover, it was also possible to use continuous morphological traits to locate in a molecular phylogeny, those specimens for which no DNA sequences were obtained, such as the holotype of *X. australis*.

In Chapter 4 the taxonomic identity of the representative genome of *Schizopora paradoxa* strain KUC8140, current name *Xylodon paradoxus* is assessed. After the analyses of three DNA regions (ITS, LSU and rpb2) it is concluded that this strain corresponds to *Xylodon ovisporus*.

In Chapter 5 a new species from Cape Verde archipelago, *Xylodon jacobaeus* sp. nov., is described using molecular and morphological data.

Conclusions

1- Species recognition criteria used in the study of *Xylodon* diversity significantly affect the species delimitation, and therefore the number of species that can be distinguished; as well as their distribution patterns. Characters seldom used, such as bioclimatic preferences, can be helpful to distinguish among taxa in *Xylodon*.

2- A multilocus multispecies coalescent approach is useful to contrast among different hypotheses of species arrangements. In addition, the combination of multiple and complementary sources of taxonomic evidence (morphological, ecological, molecular, etc.) allows for more robust species delimitation in *Xylodon*.

3- Quantitative morphological characters can be used to locate a sample within a molecular phylogeny using a maximum likelihood framework through the *locate.yeti* function from the *phytools* R package. This methodology can help to solve taxonomic issues when used to assign a specimen to a specific clade.

4- Despite the recent interest in *Xylodon*, its diversity is still unknown and further studies are required to understand the evolutionary processes that generate this diversity and distribution. Poorly explored regions are a source of undiscovered fungal species, but also natural collections in museums and herbaria play a major role in preserving and providing specimens of taxa to be described.

Resumen

Introducción

Los hongos son organismos clave dada la importancia de los procesos ecosistémicos en los que participan, desde el reciclado de nutrientes hasta las asociaciones simbióticas, y se encuentran entre los que se suponen más numerosos, tras los insectos. A pesar de ello, su diversidad es una de las menos conocidas; se estima que se han descrito, hasta la fecha, entre un 3 y un 8% de las especies que se consideran probables. El estudio de la diversidad fúngica ha estado tradicionalmente sujeto al empleo de caracteres morfológicos para la delimitación de sus especies. Sin embargo, en las últimas décadas, con el desarrollo de nuevas herramientas para la obtención de datos moleculares, las secuencias de ADN han demostrado ser un poderoso aliado a la hora de delimitar y clasificar las especies. Esto se debe a que los cambios en las secuencias génicas generalmente ocurren y pueden detectarse antes que los cambios en otros caracteres, tales como los morfológicos, lo cual ha permitido desenmascarar multitud de especies que permanecían ocultas bajo un único nombre. Aunque el uso de secuencias de ADN para delimitar de especies se ha popularizado, especialmente la región ITS del ADN ribosómico nuclear (el código de barras genético o barcode de hongos), actualmente existe consenso en la necesidad de utilizar una taxonomía integradora que combine múltiples y complementarias fuentes de evidencia en la delimitación de especies.

Hasta hace unos años, el género *Schizopora* (Basidiomycota, Hymenochaetales) incluía un grupo limitado de especies de hongos corticioides cosmopolitas y descomponedores de madera, en la que causan podredumbre blanca, caracterizado por la presencia de un himenóforo poroide, con poros irregulares que se rompen al crecer y dan lugar a dientes desiguales. Estudios moleculares recientes demostraron que *Schizopora* formaba parte de *Xylodon*, uno de los géneros de hongos corticioides más extensos, con más de 80 especies descritas. Solo en los últimos 10 años, se han descrito 14 nuevas especies y se han propuesto 59 combinaciones, lo que refleja que la taxonomía del género está lejos de poder considerarse, por completo, conocida. Esta inestabilidad taxonómica, combinada con la distribución supuestamente cosmopolita de muchas de sus especies, hacen del género *Xylodon* un buen candidato para analizar las consecuencias que puede traer consigo una taxonomía no integradora, en el estudio de la diversidad fúngica.

Objetivos y resultados

La hipótesis de trabajo de esta tesis doctoral es que las aproximaciones y los caracteres utilizados en el estudio de la diversidad fúngica afectan a los resultados obtenidos en términos de: número de especies, distribución de las mismas y caracteres diagnósticos. Para corroborar o refutar esta hipótesis se seleccionaron una serie de especies del género *Xylodon* y se plantearon tres objetivos: A) evaluar cómo afecta la selección de las fuentes de evidencia taxonómica al número detectable de especies candidatas y a la distribución atribuida a las mismas; B) buscar aquellas nuevas fuentes de evidencia taxonómica que permitan delimitar mejor las diferentes especies; y C) investigar las metodologías disponibles para integrar diferentes fuentes de evidencia en la delimitación de especies.

La tesis doctoral se ha estructurado en cinco capítulos que abordan los objetivos previamente descritos, y ha obtenido los siguientes resultados:

En el Capítulo 1 se explora el efecto de la incertidumbre taxonómica a la hora de producir modelos de distribución en tres especies del género *Xylodon*. Se comprueba que los modelos realizados a partir de las identificaciones proporcionadas en las etiquetas de los especímenes y de las secuencias almacenadas en repositorios públicos dibujan distribuciones cosmopolitas. Sin embargo, cuando la misma muestra se sometió a un estudio molecular, se identificaron hasta 19 especies candidatas que, por lo general, presentaban patrones de distribución regionales o restringidos.

En el Capítulo 2 se aborda el estudio pormenorizado del complejo *Xylodon* raduloides bajo el prisma de un análisis de tres fuentes de evidencia taxonómica diferentes: moleculares, morfológicas y ecológicas. Este estudio permite desenmascarar, en el complejo, cuatro especies diferentes cada una de ellas con un patrón geográfico muy marcado: *Xylodon laurentianus* sp. nov. para América del Norte, *X. novozelandicus* sp. nov. en Australia-Nueva Zelanda, *X. patagonicus* sp. nov. para la región Patagónica y *X. raduloides* con distribución Europea.

En el Capítulo 3 se analiza a fondo la taxonomía del complejo de *Xylodon australis* a partir de datos moleculares y morfológicos; además, se evalúa el potencial de una aproximación basada en máxima verosimilitud, que permite la integración de estos dos tipos de datos. Gracias a este estudio se identifican dos especies diferentes bajo el nombre *X. australis* y se aclaran las relaciones de parentesco entre ellas. Se logra también ubicar en una filogenia molecular, usando caracteres morfológicos, aquellos especímenes que no fue posible secuenciar, como el holotipo de *X. australis*.

En el Capítulo 4 se desvela la correcta identificación de la cepa KUC8140 procedente de Corea del Sur, identificada como *Schizopora paradoxa* (actualmente *Xylodon paradoxus*) y utilizada para la secuenciación del genoma completo de esta especie. Tras el análisis de tres regiones de ADN (ITS, LSU y rpb2) se concluye que esta cepa se trata, en realidad, de *Xylodon ovisporus*.

En el Capítulo 5, por último, se describe sobre la base de datos morfológicos y moleculares *Xylodon jacobaeus* como una especie nueva únicamente conocida, hasta el momento, del archipiélago de Cabo Verde.

Conclusiones

1- Los criterios empleados en el reconocimiento de especies afectan significativamente al estudio de la diversidad de *Xylodon*, en lo que respecta a la delimitación de sus especies, al número de las mismas y a sus patrones de distribución. Caracteres hasta ahora poco utilizados en el análisis de la diversidad fúngica, como las preferencias bioclimáticas, pueden ser útiles para distinguir linajes en *Xylodon*.

2- La aproximación basada en la teoría de coalescencia utilizando múltiples regiones de ADN es una herramienta adecuada para comparar diferentes hipótesis de delimitación de especies. Además, la combinación de múltiples y complementarias fuentes de evidencia taxonómica (morfológicas, moleculares, ecológicas, etc.) ayuda a una delimitación más robusta de las especies en *Xylodon*.

3- Los caracteres morfológicos cuantitativos pueden ser usados para ubicar los especímenes en una filogenia molecular a través de un enfoque de máxima verosimilitud utilizando la función *locate.yeti* implementada en paquete *phytools* del lenguaje R. Esta metodología puede ayudar a resolver los problemas taxonómicos que surgen al intentar asignar especímenes a un clado determinado cuando no se dispone de datos moleculares.

4- A pesar del reciente interés mostrado en el estudio de *Xylodon*, su diversidad es aún desconocida y se requieren futuros estudios para comprender los procesos evolutivos que han generado tal diversidad y la distribución de sus especies. Las regiones poco exploradas del planeta son una fuente de especies fúngicas aún por descubrir, pero las colecciones de los museos y herbarios juegan también un papel crucial al conservar especímenes ya colectados de un buen número de táxones aún por describir.

List of Manuscripts

This dissertation is based on five manuscripts written for publication in international scientific journals. The title, authors and state of publication of each manuscript are specified below.

Chapter 1. Fernández-López J, Telleria MT, Dueñas M, Martín MP. Accounting for taxonomic uncertainty in fungal species distribution models: the case of *Xylodon*. Manuscript in preparation for submission to Fungal Ecology.

Chapter 2. Fernández-López J, Telleria MT, Dueñas M, Wilson AW, Padamsee M, Buchanan PK, Mueller GM, Martín MP 2019. Addressing the diversity of *Xylodon raduloides* complex through integrative taxonomy. IMA Fungus 10:2. https://doi.org/10.1186/s43008-019-0010-x (5-year Impact Factor 4.19)

Chapter 3. Fernández-López J, Telleria MT, Dueñas M, Laguna M, Schliep K, Martín MP. New tools for old problems: the case of *Xylodon australis*. Manuscript in preparation for submission to Scientific Reports.

Chapter 4. Fernández-López J, Martín MP, Dueñas M, Telleria MT 2018. Multilocus phylogeny reveals taxonomic mis-identification of the *Schizopora paradoxa* (KUC8140) representative genome. MycoKeys 38: 121–127. https://doi.org/10.3897/mycokeys.38.28497 (5-year Impact Factor 2.43)

Chapter 5. Fernández-López J, Dueñas M, Martín MP, Telleria MT 2018. *Xylodon jacobaeus* sp. nov. Fungal Planet 867. In Crous et al. Fungal Planet description sheets: 785–867. Persoonia 41. https://doi.org/10.3767/persoonia.2018.41.12 (5-year Impact Factor 6.86)

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General introduction

Naming the fungal diversity: relevance and challenges

Fungi are considered key organisms across all ecosystems, mainly because of the fundamental roles they play in essential ecological processes such as wood decomposition, mycorrhizal associations, pathogenicity, etc. Moreover, they represent one of the three large eukaryotic lineages that dominate terrestrial ecosystems (Nagy et al. 2017). However, despite these dominant roles, their diversity is largely unknown. According to recent studies, the commonly cited estimate of 1.5 million fungal species is conservative. New evidence increases those estimates up to 2.2 – 3.8 million species of fungi (Hawksworth & Lücking 2017). Since currently around 120,000 fungal species have been described, this means that only around 3% to 8% of species have been described to date.

The number of species being recognized is necessarily impacted by the species concepts used, and this remains an important discussion topic for fungi (Taylor et al. 2000; Öpik & Davison 2016). It has been suggested that the same species concept is not always applicable across all fungal taxa (Giraud et al. 2008) due to the numerous and different evolutionary processes that can lead to fungal speciation and the high variation in the temporal extent of speciation among different fungal groups.

In the last decade, around 1,800 species have been described per year. This represents a slight increase to the described species of previous decades (1,300 species described annually), mainly due to advances in the use of molecular tools (Hawksworth & Lücking 2017). Nevertheless, in contrast to other organisms, the accumulation curve of new species descriptions are far from stabilizing for fungi (Mora et al. 2011).

Where to look for this hidden fungal diversity is a topic of interest for fungal researchers (Hawksworth & Rossman 1997). Poorly explored habitats and biodiversity Hot Spots, such as oceanic islands or tropical regions, may be a source of undiscovered fungal species (Hawksworth & Lücking 2017). For instance, Aptroot (2001) reported up to 200 species of ascomycetes in a single *Elaeocarpus* tree in Papua New Guinea, many of them new to science. This high fungal diversity related to a particular tree species is also found in other groups; thus, up to 250 species of ectomycorrhizal fungi were estimated in association with *Dicymbe corymbosa* in a forest in Guyana (Henkel et al. 2012). Other geographical areas like tropical Africa or very specific and poorly explored habitats such as mammal and insect guts are just some examples where fungal diversity may be hidden.

However, it is also argued that a large number of undiscovered species have already been collected and is awaiting modern studies to reveal their diversity. Bebber et

al. (2010) estimate that of the 70,000 species of flowering plants to be described, more than a half would be stored in herbaria collections. For fungi, conservative estimates speculate that around 20,000 new species may be already collected and stored in herbaria, awaiting formal description (Hawksworth & Rossman 1997), although this number is probably underestimated. In this context, collections in herbaria and museums are an essential source of data for the study of biodiversity, and there is a need for reevaluation.

One reason for this reevaluation is the shift from the morphological to the phylogenetic species concept. Traditionally, species delimitation was based mainly on morphological and cultural features. From the 1990's the incorporation of molecular data has led to a redefining of species concepts (Taylor et al. 2000). The revaluation of known taxa under this view is unveiling a high diversity masked under a single species name and it has shown that the number of fungal species has been underestimated for a long time. One example of this situation is the decay fungus *Schizophyllum commune*. Although initially this complex was described as a single widely-distributed species, DNA analyses confirmed the existence of several phylogenetic species with a regional distribution (Taylor et al. 2006). In fact, this shift from a single cosmopolitan species to a several locally distributed species is common in many Basidiomycota (Petersen & Hughes 1999). Cai et al. (2014) identified 12 new species of lethal amanitas in a group of 28 phylogenetic species, demonstrating that the diversity of this fungal group was underestimated despite their importance to public health. It has been suggested that the number of fungal species would rise by a factor of five or more in some groups, with the molecular study of species complexes (Hawksworth & Rossman 1997). Some extreme cases, such as the basidiolichen *Cora glabrata* have been shown to contain up to 126 species under a single name (Lücking et al. 2014).

In this context, as new techniques are modifying fungal systematics, updating the information about herbaria and museum collections becomes fundamental to avoid misleading inferences about fungal diversity or biogeography (Smith et al. 2016). Wrong identifications in fungal biodiversity databases could lead not only to inaccurate

knowledge of nature, but also to important economic losses or conservation and public health issues (Bortolus 2008; Fernández-López et al. 2018a).

Nowadays, it is commonly accepted that a polyphasic and an integrative approach should be taken in order to delimit and describe new species, combining different sources of evidence such as morphological, ecological and molecular traits (Dayrat 2005; Quaedvlieg et al. 2014). The application of this framework in the study of fungal diversity could be a challenge in many ways, due to the complexity of fungal life cycles, the diversity of habitats they occupy and the little attention that has been traditionally lent to some groups, among other reasons. However, the use of new techniques and tools to explore the fungal tree of life is a requirement to obtain a true view of the dimension of this group of organisms.

Approaches in the study of fungal diversity

Traditional morphological methods

The study of fungal diversity has been traditionally dominated by the use of morphological characters for species delimitation and classification (Giraud et al. 2008). This method was largely applied across all fungal taxa. It consists in the selection of homologous morphological structures, either macroscopic or microscopic, and comparison among species to establish kinship relations based on similarities or differences in such traits. However, homologous structures are not always easy to identify across all taxonomic levels, and their similarities are often due to convergent adaptations rather than evolutionary relationships e.g. resupinate morphology of corticioid fungi (Larsson et al. 2004). Moreover, even when homologous characters are well known, they often presents high variability, and is not always clear when those differences are enough to split or lump the species (Petersen & Hughes 1999). In general, recent studies have demonstrated that much fungal diversity could be masked by a morphological species recognition approach (MSR), since morphological differences usually appears later than genetic evidence in the speciation process (Taylor et al. 2000). This situation could be especially frequent when the species grow on stable habitats such as wood-inhabiting fungi, promoting morphological stasis and therefore cryptic speciation (Mueller et al. 2001).

Mating compatibility studies

In many cases it is complicated to discern morphological differences between fungal species. Fruit bodies of many fungal species are often ephemeral and their reproductive structures have been scarcely collected (Boidin 1986). For this and other reasons, mycologists have benefited from the characterization of fungal cultures and mating compatibility tests since the early 20th century such as the studies of Bensaude (1918) in basidiomycetes. Mating compatibility studies rely on the biological species concept in order to differentiate species (biological species recognition, BSR), considering that two individuals belong to the same species if they are able to produce, always and indefinitely, fertile descendants between them (Vandendries 1923). This methodology was broadly used especially in wood decaying fungi due to their intrinsic characteristics related to germination, development and culture conservation (Nobles 1965; Stalpers 1978; Boidin 1986). Although it was still used until the early 21th century (Hallenberg 1983; Hallenberg et al. 1996; Paulus et al. 2000), this concept has been heavily criticized not only because it fails to accommodate asexual phases of some species, but also because it is demonstrated that relatively distant species which differ in morphology and ecology may be able to originate fertile offspring (Natvig & May 1996). Furthermore, mating tests produce a high rate of false negatives (Petersen & Hughes 1999). Nowadays, given the accessibility of alternative approaches that are not too expensive and offer better results such as the molecular tools, mating compatibility studies are not common in fungal species delimitation literature.

The rise of the molecular era

From the 1990s onward, molecular tools arose as a powerful approach in the study of fungal diversity. Molecular methods revealed hidden species masked under a single species name because changes in gene sequences usually occur and can be detected before changes in other traits, such as morphological characters (Taylor et al. 2000). Thus, DNA sequences were considered as useful characters to arrange or lump species, and the best way to delimit fungal species consistent with an evolutionary view. In the last decades, molecular data and mathematical approaches to analyze them have deeply changed the traditional and mainly morphological fungal taxonomy.

In the first studies, a few or even a single DNA region was used in molecular

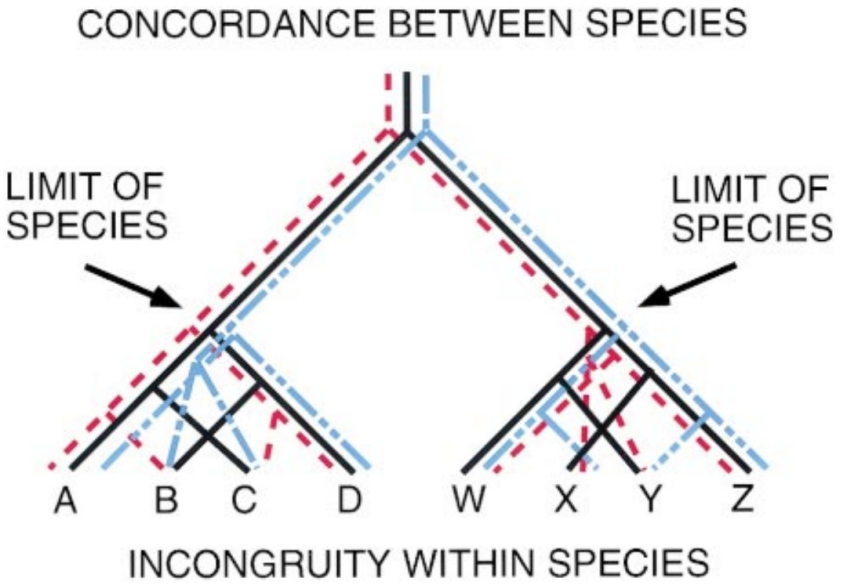

Fig 1. Simultaneous analysis of three gene genealogies shows how the transition from concordance among branches to incongruity among branches can be used to diagnose species. From Taylor et al. (2000).

analyses to differentiate species (usually the nuclear-encoded ribosomal RNA cistron), but the number of DNA regions used for phylogenetic reconstruction increased rapidly. Soon, researchers realized that different DNA regions could have different evolutionary histories. This is usually due to evolutionary processes that can generate discordance among gene trees, such as incomplete lineage sorting (Stewart et al. 2014). A taxonomic milestone was the introduction of the phylogenetic species concept in fungi (PSC) published by Taylor et al. (2000). This study explains a methodology to apply the PSC called the genealogical concordance phylogenetic species recognition (GCPSR). When multiple and independent loci are used for species delimitation, individual gene trees are constructed and different species can be recognized at the point at which there is a transition from concordance among gene trees to incongruity between them (Fig 1). Nowadays, there are many methods for species delimitation that, based on coalescent theory, are able to take into account discordance among gene trees and species trees (Mallo & Posada 2016). Although its use has become widespread for other organisms such as plants or animals, its application in fungi is still scarce (Geml et al. 2006; Martín et al. 2018).

With the generalization of molecular tools in species delimitation, researchers searched for those DNA regions with the best trade-off between sequencing effort and resolving power for discrimination between closely related species. According to Kress & Erickson (2008), the denominated barcoding region "*must satisfy three criteria: (i) contain*

significant species-level genetic variability and divergence, (ii) possess conserved flanking sites for developing universal PCR primers for wide taxonomic application, and (iii) have a short sequence length so as to facilitate current capabilities of DNA extraction and amplification". Schoch et al. (2012) tested six DNA regions in order to determine a universal DNA barcode marker for fungi. As another milestone, the nuclear ribosomal internal transcribed spacer (ITS) was formally proposed as the primary fungal barcode marker because of its high probability of successful identification for the broadest range of fungi (Schoch et al. 2012). From this point, many studies have used the fungal barcode to directly distinguish species or operational taxonomic units (OTUs) using either phylogenetic analyses (gene trees) or barcoding gap approaches (barcoding gap species recognition BGSR). This has been especially useful for massive sequencing of environmental samples e.g. soil fungi (Tedersoo et al. 2014). However, it is currently accepted that a unique DNA region could be not enough for fungal species delimitation. Despite the discrimination power of ITS, taxonomic/systematic studies can benefit by including other genetic regions (Balasundaram et al. 2015). The translation elongation factor 1-α (TEF1α) has been recently described as a possible secondary fungal barcode (Stielow et al. 2015), but a multi-locus approach is still recommended to ensure a robust molecular diagnostic of fungal species.

Beyond the molecule

Despite the success of molecular tools in the study of biodiversity, taxonomists and evolutionary biologists have recognized in the last decade the importance of including multiple disciplines for species delimitation (de Queiroz 2007). This integrative taxonomy highlights the importance of merging traditional morphological studies with other sources of evidence such as molecular or ecological traits to support species hypothesis (Dayrat 2005). This poliphasic approach is especially useful in those species groups where morphological and genetic diversity are not correlated (Barley et al. 2013). According to Samson & Varga (2009), a combination of multilocus sequence data, morphological measures, physiological characteristics and ecological data have been considered as a "gold standard" for fungal species delimitation.

Environmental and bioclimatic preferences have been successfully used in other organisms for species identification (Rissler & Apodaca 2007; Raxworthy et al. 2007). These traits are usually useful because, according to Wiens & Graham (2005), the

adaptation to a specific ecological niche plays a key role in processes such as allopatric speciation and therefore they are closely linked. Also, the influence of climate and environmental factors in fungal community structure and distribution has been demonstrated (Wollan et al. 2008; Fukasawa & Matsuokab 2015), but ecological or environmental traits are not commonly applied for fungal species delimitation despite their importance (e.g. Gazis et al. 2011). The inclusion of ecological traits in an integrative taxonomy framework could be a useful tool for fungal taxonomy especially in those groups where morphological stasis has resulted in "cryptic" evolutionary radiations (Mueller et al. 2001).

As a last challenge to account for multiple sources of evidence in taxonomy and systematics, some recently developed methodologies integrate molecular and morphological data in a single framework. Specifically, several approaches have been described to include fossils or recently extinct taxa in molecular phylogenies using morphologically continuous traits (Revell et al. 2015; Parins-Fukuchi 2018). This includes those specimens for which it is impossible to obtain DNA sequences, either for methodological (damaged DNA) or historical constrains (type collections with an intrinsic historical value). These methodologies could be powerful tools to support the phylogenetic location of new fungal species and to avoid nomenclatural issues.

The study case: from *Schizopora* to *Xylodon*

Velenovský (1922) described the genus *Schizopora* with *Polyporus laciniatus* Velen. as species type. Almost 20 years later, Pilát (1941) synonymized *Polyporus laciniatus* Velen. to *Hydnum paradoxum* Schrad per Fries. The genus was largely neglected until Donk (1960) rescued it. Donk (1967) emended the genus *Schizopora*, providing a detailed list of its morphological traits and including *Schizopora paradoxa* (Schrad.) Donk as the unique species.

According to Donk (1967), *Schizopora* was characterized by "*Fruitbody effused, annual, whitish to cream; context toughish; hymenophore tubulose to irpicoid, the pores medium-sized. Context dimitic by skeletals; generative hyphae thin-walled, remaining distinct, with clamps at the septa, non-inflating; skeletals firm to thick-walled, those in the trama of the dissepiments or flattened teeth slightly spindle-shaped, not bending into the hymenium, the walls hyaline, somewhat congophilous. Hymenial leptocystidia present; thin-walled hyphal ends building up the growing edge of the dissepiment encrusted by spaced, small crystal bodies. Basidia often slightly constricted in the middle (utriform), 4-spored. Spores ovoid, adaxially only slightly flattened, small (4-6 µ long), colourless: walls thin, smooth, non-amyloid*".

Schizopora was included in the Polyporaceae (Donk 1964, 1967; Ryvarden 1978; Ryvarden & Johansen 1980). Jülich (1982) located *Schizopora* in the new Schizoporaceae family. It was at first considered close to *Hyphodontia* (Donk 1967), a genus included in Corticiaceae, and the cultural characterization supported this relationship (Hassan & David, 1983). In this way, the connection between *Schizopora* and other genera such as *Hyphodontia* or *Fibrodontia* was also reported by Eriksson & Ryvarden (1984) due to their similar microscopic characteristics.

With the rise of DNA sequencing techniques and their use in phylogenetic reconstructions, fungal classifications were rearranged, and the connection between *Schizopora* and *Hyphodontia* was early confirmed in general studies (Hibbet et al. 1997). Larsson et al. (2006) conducted a phylogenetic study of the hymenochaetoid clade using 5.8S and nucLSU rDNA sequences, and definitively located *Schizopora* close to *Hyphodontia* and *Coltricia* species in the Hymenochaetales (Fig 2).

Hjortstam & Ryvarden (2007) reintroduced the genus *Xylodon* adding some combinations that were complemented in Hjortstam & Ryvarden (2009). Later, Riebesehl

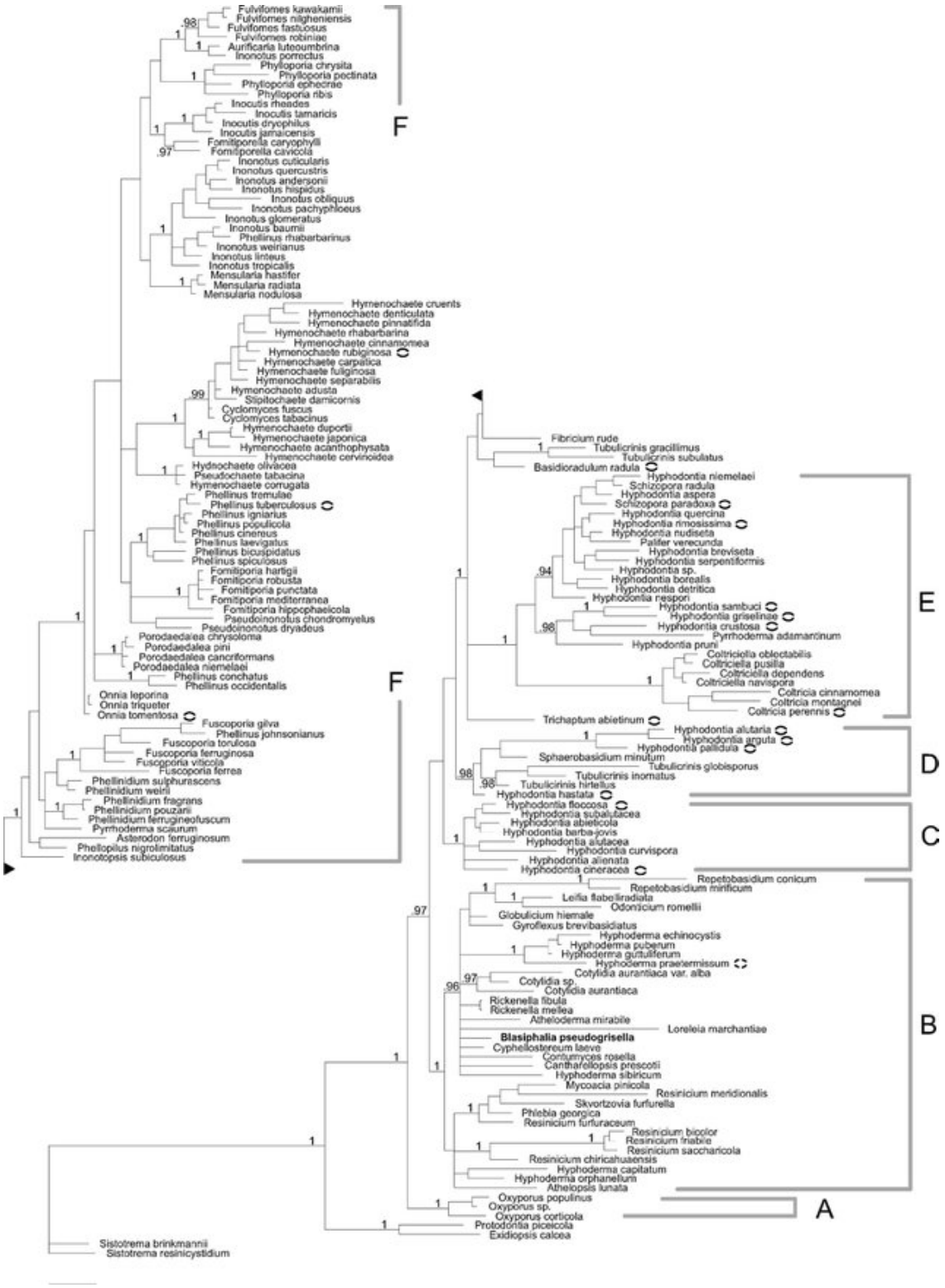

Fig 2. Phylogenetic relationships of Hymenochaetales inferred from 5.8S and LSU nrDNA sequences with Bayesian analysis (posterior probabilities are shown above internodes). Closed horizontal parentheses indicate that the species has dolipores with continuous parenthesomes. Broken horizontal parentheses indicate presence of the perforated parenthesome type. Clades: A. *Oxyporus* B. *Rickenella* C. *Kneifiella* D. *Hyphodontia* E. *Coltricia* F. Hymenochaetaceae. From Larsson et al. (2006).

& Langer (2017) reported a broad study about *Hyphodontia s.l.* using both molecular and morphological data, which included many of the species traditionally classified in *Schizopora*, *Xylodon* and other genera. In this study, they found no support to maintain the *Schizopora* genus, since phylogenetic analyses using two nuclear DNA regions (ITS and LSU) revealed that *Xylodon* and *Schizopora* species were completely mixed in a single big clade (Fig 3).

Since the *Xylodon* name is older, it preserved the nomenclatural priority according to Turland et al. (2018) and all the species traditionally included in *Schizopora* were absorbed by *Xylodon*.

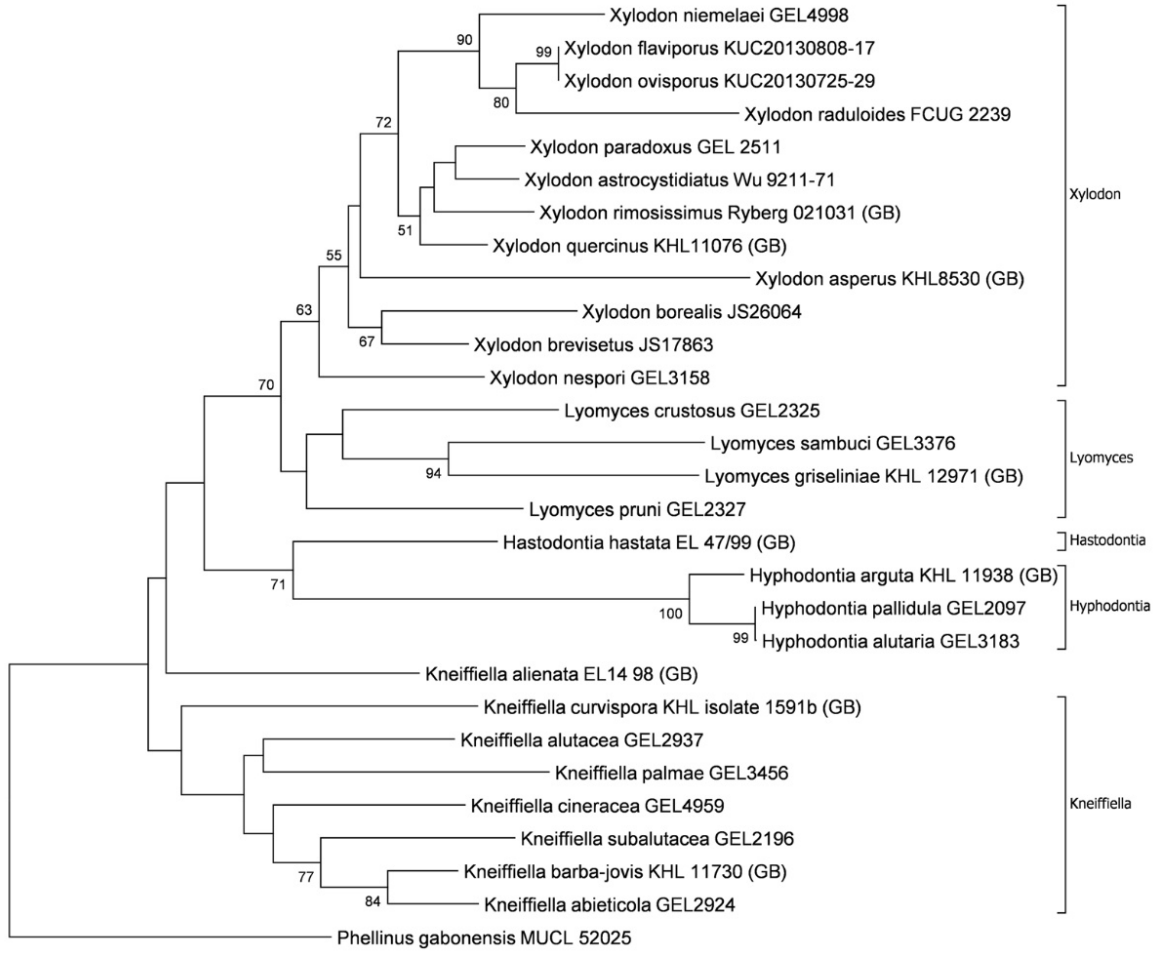

Fig 3. Phylogram for *Xylodon*, *Lyomyces*, *Hastodontia*, *Hyphodontia* and *Kneifiella* clades based on nrLSU sequences using the minimum evolution method. Bootstrap test percentages are shown next to the branches. From Riebesehl & Langer (2017).

According to Riebesehl & Langer (2017), *Xylodon* (Pers.) Gray emend. Riebesehl & E. Langer is characterized by: "*Basidiome resupinate. Hymenophore smooth, tuberculate, grandinioid, odontioid, coralloid, irpicoid or poroid. Hyphal system monomitic, pseudodimitic, dimitic or trimitic. Hyphae with clamp connections. Cystidia present or absent, different types: bladder-like, bottleped, capitate, clavate, cylindrical, fusiform, lecythiform, moniliform, pyriform, subulate cystidia, astro- or gloeocystidia. Basidia barrel shaped, clavate, cylindrical, pyriform, sinuous or urniform, with two or four sterigmata. Spores ellipsoid, suballantoid, cylindrical, ovoid or subglobose, smooth, thin- or thick-walled, hyaline, inamyloid, acyanophilous or slightly cyanophilous*".

Xylodon is considered a cosmopolitan genus of white-rot fungi, growing on angiosperms as well as gymnosperms. According to Hjortstam & Ryvarden (2007, 2009) *Xylodon* is one of the largest genera of wood-rotting fungi, with 172 current legitimate names (http://www.mycobank.org, December 2019; Crous et al. 2004). During the last ten years, 14 new *Xylodon* species have been described (Spirin & Miettinen 2015; Chen et al. 2018; Fernández-López et al. 2018b; Viner et al. 2018; Riebesehl et al. 2019; Fernández-López et al. 2019; Shi et al. 2019), 59 combinations were proposed (Hjortstam & Ryvarden 2009; Riebesehl & Langer 2017; Chen et al. 2018) as well as two new names (Hjortstam & Ryvarden 2009; Riebesehl & Langer 2017). In addition to the 77 species formally recognized by Riebesehl et al. (2019) based on molecular and morphological data, four more species have been recently described (Fernández-López et al. 2019; Shi et al. 2019). This shows that *Xylodon* taxonomy is far from being completely known, and new data from different sources may shed light on its diversity. This taxonomic uncertainty combined with the wide geographical distribution of many species, makes *Xylodon* a good candidate to study the consequences of misleading taxonomy, including the biogeographic patterns inferred for their species, conservation problems or economic impact (Bortolus 2008).

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Hypothesis, objectives and thesis structure

Recent studies have revealed a great internal diversity for the corticioid fungi *Xylodon* (Riebesehl & Langer 2017). The rearrangement of species based on molecular evidences has shown that traditional morphological characters used for species classification failed in establishing evolutionary relationships. This highlights the need of search for additional evidences to understand *Xylodon* complexity.

Our working hypothesis is that the characters and approaches used in the study of *Xylodon* diversity strongly affects to the results obtained in terms of number, distribution and diagnostic traits of species. To test this hypothesis, morphological, molecular and ecological analyses are conducted over a group of selected species in *Xylodon*. Particularly, we address the following objectives: A) to evaluate how taxonomic uncertainty could affect to the number of candidate species and their distribution patterns (chapters 1, 2, 3, 4); B) to look for new sources of evidence in order to better recognize different species in *Xylodon* (chapters 2, 4); and C) to investigate the available methodologies to combine different evidence sources (morphological, molecular, ecological and others) for the identification of *Xylodon* species (chapter 3, 5).

This PhD thesis dissertation is structured in five chapters to address the objectives above mentioned.

- Chapter 1. We explore the effect of taxonomic uncertainty and wrong identifications in the determination of species candidates and their distribution using species distribution models (SDMs). We focus specifically in differences obtained from morphological vs molecular species recognition criterion of three widely distributed morphospecies from the corticioid genus *Xylodon*. Number of candidate species and the main factors that explain their distributions were compared for each recognition criterion. We also analyze the biogeographical hypothesis supported by each criterion and their implications for conservation.

- Chapter 2. In the second chapter, we address the study of *Xylodon raduloides* complex through an integrative taxonomy framework. Specifically, our aim is to achieve a comprehensive understanding of the taxonomic diversity of the complex through the use of multi-locus species coalescent phylogeny, morphological characters, and environmental equivalence analysis. We evaluate if different traits as environmental requirements can be used to support molecular data to distinguish between *Xylodon* species.

- Chapter 3. This chapter investigates the available methodologies to combine different evidence sources in the study of fungal diversity. A maximum likelihood framework was used to locate samples with no molecular data available into a molecular phylogeny. We evaluate the potential of this method to be used with historical collections for which destructive sampling to obtain molecular data is not allowed. We apply the methodology to the case of *Xylodon australis* complex in order to solve different taxonomic issues.

- Chapter 4. The fourth chapter assesses the taxonomic identity of the representative genome of *Schizopora paradoxa* strain KUC8140, current name *Xylodon paradoxus*. We used three DNA regions (two non protein-coding and one protein-coding) to compare KUC8140 strain to other *Xylodon* species using a multilocus approach.

- Chapter 5. Finally, the fifth chapter describes the new species *Xylodon jacobaeus* J. Fernández-López, M. Dueñas, M.P. Martín & Telleria using molecular and morphological characters. We study its relationships to other *Xylodon* species and describe the most relevant diagnostic characters based on micromorphological traits.

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Chapter 1

Accounting for taxonomic uncertainty in fungal species distribution models: the case of *Xylodon*

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ABSTRACT

Species distribution models based on climatic or other environmental predictors are useful to explain a species geographic range. For many groups of organisms, including fungi, the increase of occurrence data sets has generalized their use. However, fungal species are not always easy to distinguish, and taxonomy of many groups is not completely settled. This study explores the effect of taxonomic uncertainty in databases used for modeling fungal distributions. We analyze distribution models for three morphospecies from the corticioid genus *Xylodon* (Hymenochaetales, Basidiomycota), comparing models based on species names in herbarium and sequence vouchers with models derived from species identified by DNA barcode. Differences in the contribution of predictors that drive the distribution of each modeled taxon and the extent of their ranges were studied. Records under *Xylodon paradoxus*, *X. flaviporus* and *X. raduloides* were obtained from herbarium collections and GenBank repository. Two grouping criteria were used: (1) specimens were grouped by their collection or sequence voucher names and (2) specimens were grouped following molecular identification using ITS sequences (the fungal barcode) through barcoding gap species recognition (BGSR). Climatic, geographic and biotic variables were used to predict the potential distribution of each taxon through MaxEnt algorithm. From the three morphospecies selected according to voucher names, up to 19 species candidates were detected using BGSR. Climatic variables were the most important predictors in the distribution models made from names in herbarium and sequence vouchers, but its importance decreased when BGSR was applied. In general, the extent of species distributions was more restricted for taxa under BGSR. Our results show that taxonomic uncertainty has a strong effect in *Xylodon* species distribution models. Misleading results can be obtained when cryptic species or identification errors mask the actual diversity of the presence records. Preserved specimens in natural history collections offer the possibility to assess if the species name in labels matches the current species recognition criteria.

INTRODUCTION

In recent decades, ecological and biogeographical studies have become popular due to new tools for modeling species distributions from presence records (Elith et al. 2006). These approaches, based on correlations between species occurrences and environmental predictors, have been used to obtain maps of potential distributions for poorly studied species, to evaluate pest risks (Sutherst 2014) or to help in the design of natural reserves (Watts et al. 2009). The combination of powerful, new algorithms with the increase of environmental cartography have made it possible to apply these methodologies in a broad range of organisms, including different groups of fungi such as ectomycorrhizal (Wolfe et al. 2010) or soil biocrust (Belnap et al. 2014). Indeed, fungi have been pointed as one of the most benefited groups due to the large number of occurrence records stored in herbarium collections (Wollan et al. 2008; Hao et al. 2020).

In the modeling process, much attention has been paid in algorithm performance (Qiao et al. 2015) to the accuracy of predictor variables (Petitpierre et al. 2017), and the sample size and collection bias (Beck et al. 2014; Fourcade et al. 2014), but taxonomic uncertainty in presence records has attracted less interest (Elith et al. 2013). This could be due in part to the difficulty of assessing the reliability of records in public repositories or citizen science databases (Lozier et al. 2009). On many occasions only a list with geographic coordinates is available, and researchers must rely on the accuracy of geographic coordinates and species identifications. This taxonomic uncertainty could produce misleading results with important conservation or economic consequences (Bortolus 2008). This issue plays a major role in those groups for which taxonomy is not completely resolved, or organisms that require expertise to correctly identify the species (Smith et al. 2016).

One of the most important sources of taxonomic uncertainty in public repositories is the shift in species recognition criteria in recent decades (Bridge et al. 2003). The traditionally applied morphological species recognition, MSR (Taylor et al. 2000), has been used to identify more than 70,000 fungal species (Hawksworth et al. 1996; Taylor et al. 2000), resulting in a worldwide distribution for many of these taxa (Hallenberg 1991). This homogeneous distribution for many fungal species has supported the Baas Becking hypothesis: "*Everything is everywhere, but environment selects*" (Baas Becking 1934). This idea, originally applied to microorganisms, has been extended to include fungal species due the small size of fungal spores, the main agent of fungal dispersion (Taylor et

al. 2006). The apparent unlimited dispersal ability of many fungal species has often been used to explain their cosmopolitan distributions (Davison et al. 2015). Nowadays, the development of molecular tools has allowed identification of a significant amount of hidden biodiversity, cryptic or sibling species, masked under a single species name (Koufopanou et al. 1997; Fišer et al. 2018). The shift from morphological to phylogenetic species recognition, PSR (Taylor et al. 2000) has redrawn the map of fungal distribution, and new biogeographical patterns have arisen when morphospecies were redefined following PSR criteria. A single species with worldwide distribution has been redescribed as several species with regional or restricted distribution (Nilsson et al. 2003; Telleria et al. 2010; Carlsen et al. 2011). This new approach in the study of fungal diversity has promoted the idea that cosmopolitanism in fungi is just the result of the application of MSR, rather than an actual biodiversity distribution pattern (Sato et al. 2012). In this context, natural historical collections or DNA sequence repositories allow for a reevaluation of the species names assigned in collections or sequence vouchers, and therefore, the assessment of the effects of taxonomic uncertainty or misleading specimen identifications in the potential distribution inferred by species distribution models.

Xylodon (Hymenochaetales, Basidiomycota) is a white-rot fungus considered one of the most species rich corticioid genera (Hjortstam & Ryvarden 2007, 2009), and plays an important role as a wood decomposer from temperate to tropical forests. It contains many species that have been traditionally cited worldwide and its taxonomy has rapidly changed in recent years (Riebesehl & Langer 2017). In addition, despite their macroscopic basidiocarps, the morphological traits used to distinguish among closely-related species are highly homoplasic, making them prone to errors in specimen identifications.

The aim of the present study was to analyze the effect of taxonomic uncertainty and misidentifications in natural collections and sequence labels on species distribution models in *Xylodon*. We analyze the possible effects in two ways: first, we assessed if a greater hidden diversity could be masked under a single species name in vouchers using the ITS DNA region and barcoding gap analysis (Schoch et al. 2012; Puillandre et al. 2012); second, we constructed species distribution models following both identification criteria (names in vouchers; species candidates obtained from barcoding gap analysis) and analyzed differences in the contribution of predictor variables and the distribution area sizes.

MATERIALS AND METHODS

Species studied and selection of material

A general search of preserved specimens in the Global Biodiversity Information Facility (GBIF) confirmed the worldwide distribution of presence records assigned to these three morphospecies: *Xylodon flaviporus*, *X. paradoxus* and *X. raduloides* (Fig 1). These three *Xylodon* species traditionally known as being widely distributed were selected to discuss the effect of taxonomic uncertainty on biogeographical hypotheses supported by species distribution models. Those species have been traditionally located in *Schizopora*, but recent studies demonstrated that it is not possible to separate *Schizopora* from *Xylodon* on molecular basis (Riebesehl & Langer 2017) and therefore, *Schizopora* species are currently integrated in *Xylodon*.

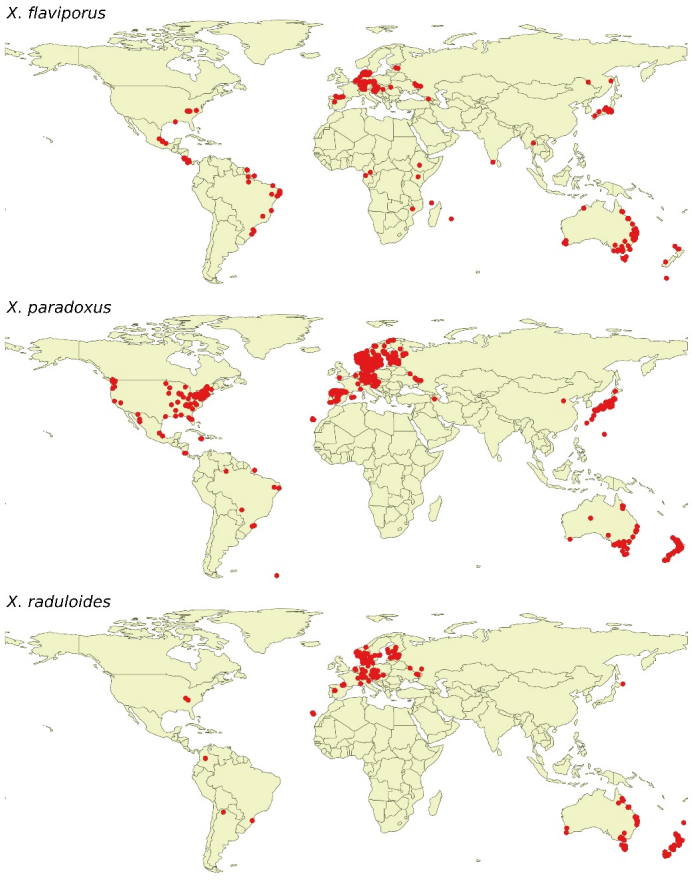

Fig 1. Presence records for three *Xylodon* morphospecies traditionally considered as widely-distributed, from the Global Biodiversity Information Facility database (GBIF). These occurrences correspond only to preserved specimens in natural historical collections. DOIs: *Xylodon flaviporus* https://doi.org/10.15468/dl.tvfuk9; *Xylodon paradoxus* https://doi.org/10.15468/dl.mwpda3; *Xylodon raduloides* https://doi.org/10.15468/dl.wkcufk.

Xylodon flaviporus (Berk. & M.A. Curtis ex Cooke) Riebesehl & E. Langer was originally described from Venezuela as *Poria flavipora* Berk. & M.A. Curtis. It has 24 synonyms according to Index Fungorum (Appendix S1) and has been reported from numerous hardwood substrates, such as *Castanea*, *Eucalyptus*, *Fagus* and *Quercus*, but also on conifers, such as *Picea* and *Pinus*. *Xylodon flaviporus* has been described as distributed worldwide (Fig 1), especially in warm and tropical zones. It has been reported from around the world: America, Africa, southern Europe and South Asia (Gilbertson & Ryvarden 1987; Paulus et al. 2000; Wu 2000; Núñez & Ryvarden 2001; Ryvarden & Melo 2014).

Xylodon paradoxus (Schrad.) Chevall. was described as *Hydnum paradoxum* Schard. from Germany, and it has 52 synonyms following Index Fungorum (Appendix S1). It occurs in deciduous woodlands, mainly in Europe (Fig 1), although it has also been reported worldwide (Ryvarden 1978; Ryvarden & Johansen, 1980; Eriksson et al. 1984; Bernicchia 2005; Ryvarden & Melo 2014).

Xylodon raduloides Riebesehl & E. Langer was originally described as *Poria radula* Pers. It was largely considered *Xylodon paradoxus*, but was split by Hallenberg (1983). It has 30 synonyms according to Index Fungorum (Appendix S1) and it has typically been associated with angiosperm wood. *Xylodon raduloides* has been reported from distant locations (Fig 1) such as Europe (Langer 1994; Ryvarden & Gilbertson 1994; Ryvarden & Melo 2014), North America (Hallenberg 1983; Gilbertson & Ryvarden 1987), South America (Langer 1994), temperate Asia (Hallenberg 1983; Langer 1994), and Australasia (Paulus et al. 2000).

All the available collections of these three morphospecies were analyzed from a total of four herbaria (Table 1). Label information was used to assign the geographic location for each record (unprojected coordinates, WGS87 datum). When an exact location was not provided, label information such as towns or kilometer points along roads was used to obtain geographic coordinates. Only records with known coordinate uncertainty of less than 5 km (i.e. those which on average could be placed in a single 10 x 10 km cell) were considered. A basidiome fragment from herbarium specimens (less than 10 mg) was removed to perform molecular analyses.

Table 1. Selected specimens and species assignation following names in herbarium and sequence vouchers (Data Set 1) and following barcoding gap species recognition (Data Set 2, Fig. 2). New sequences in bold.

Label/ Voucher species name (Data Set 1)	Country	BGSR (Data Set 2)	Collection specimen	GenBank Accession n.º
Xylodon flaviporus (Berk. & M.A. Curtis ex Cooke) Riebesehl & E. Langer				
	Brazil	SC-C5	NY 1045	**Acc. N.**
	Cameroon	SC-B1	NY s.n.	**Acc. N.**
	Cameroon	SC-C7	O-F 915884	**Acc. N.**
	Cameroon	SC-C3	MA-Fungi 38220	**Acc. N.**
	China	SC-C5	CLZhao 53	MG231630
	China	SC-C5	CLZhao 2384	MH114732
	China	SC-C5	CLZhao 60	MG231631
	China	SC-C5	CLZhao 4785	MK269030
	China	SC-C5	CLZhao 34	MH114928
	China	SC-C5	CLZhao 83	MG231632
	China	SC-C5	CLZhao 116	MG231634
	China	SC-C5	CLZhao 85	MG231633
	China	SC-C5	CLZhao 3459	MK269271
	China	SC-C5	CLZhao 3468	MK269027
	China	SC-C5	CLZhao 3194	MH114735
	China	SC-C5	CLZhao 3143	MH114733
	China	SC-C5	CLZhao 3148	MH114734
	China	SC-C5	CLZhao 3275	MK269026
	China	SC-C5	CLZhao 3609	MK269028
	China	SC-C5	CLZhao 3656	MK269029
	China	SC-C1	SWFC 001828	MK838854
	China	SC-C1	SWFC 004636	MK894105
	China	SC-C1	SWFU 001902	MK809470
	China	SC-C1	SWFC 001831	MK838888
	China	SC-C1	SWFU 001840	MK809478
	China	SC-C1	CLZhao 5850	MK343690
	China	SC-C1	Wu 0211-53	MF540763
	China	SC-C1	SWFC 001824	MK838853
	China	SC-C1	SWFC 001817	MK838856
	Costa Rica	SC-C5	O-F 507425	**Acc. N.**
	Ecuador	SC-C5	O-F 505597	**Acc. N.**
	France	SC-C5	MA-Fungi 70678	**Acc. N.**
	France	SC-C5	MA-Fungi 79438	**Acc. N.**
	Germany	SC-C5	MA-Fungi 79440	**MH260071**
	Japan	SC-C1	O-F 507446	**Acc. N.**
	Kenya	SC-C3	O-F 507471	**Acc. N.**
	Kenya	SC-C3	O-F 507406	**Acc. N.**
	Lesser Antilles	SC-C5	O-F 507388	**Acc. N.**
	Malawi	SC-C6	O-F 507478	**Acc. N.**
	Nepal	SC-C1	O-F 507433	**Acc. N.**
	Panama	SC-C5	MA-Fungi 36573	**Acc. N.**
	Panama	SC-C5	MA-Fungi 36574	**Acc. N.**
	Panama	SC-C5	MA-Fungi 36800	**Acc. N.**
	Puerto Rico	SC-C4	PR 1853	**Acc. N.**
	Reunion	SC-C2	KAS-GEL5047	MH880203
	Reunion	SC-C2	FR-0249797	MH880201

Table 1 (cont.).

Label/ Voucher species name (Data Set 1)	Country	BGSR (Data Set 2)	Collection specimen	GenBank Accession n.º
Xylodon flaviporus (Berk. & M.A. Curtis ex Cooke) Riebesehl & E. Langer	Romania	SC-C5	FCUG 1534	AF145573
	Romania	SC-C5	FCUG 1053	AF145575
	Rwanda	SC-C6	O-F 507449	**Acc. N.**
	South Korea	SC-C5	KUC20130808-17	KJ668462
	South Korea	SC-C5	KA17-0796	MK920119
	South Korea	SC-C1	SFC20180710-24	MK992840
	Taiwan	SC-C5	FP 101622	**Acc. N.**
	Taiwan	SC-C5	ICMP 13836	AF145585
	Taiwan	SC-C5	KAS-GEL3462	MH880202
	Taiwan	SC-C1	GC 1509-71	MF540761
	Thailand	SC-C1	O-F 507441	**Acc. N.**
	USA	SC-C5	DLL2011-167	KJ140665
	USA	SC-C5	DLL2011-134	KJ140637
	USA	SC-C5	DLL2011-141	KJ140642
	USA	SC-C4	HHB 9460	**Acc. N.**
	USA	SC-C4	FP 102561	**Acc. N.**
Xylodon paradoxus (Schrad.) Chevall.				
	Chile	SC-A4	21039Tell	**Acc. N.**
	Chile	SC-A4	MD15237	**Acc. N.**
	Chile	SC-A4	MD15177	**Acc. N.**
	Chile	SC-A4	MD15041	**Acc. N.**
	Chile	SC-A4	MD15040	**Acc. N.**
	Chile	SC-A4	20984Tell	**Acc. N.**
	Chile	SC-A4	MD15171	**Acc. N.**
	Chile	SC-A4	MD15042	**Acc. N.**
	China	SC-A2	CLZhao 3220	MK269041
	Finland	SC-A1	Otto Miettinen 7978	FN907912
	France	SC-A1	MA-Fungi 79441	**Acc. N.**
	France	SC-A1	MA-Fungi 70444	**Acc. N.**
	France	SC-A1	MA-Fungi 81294	**MH260072**
	Germany	SC-A1	MA-Fungi 40866	**Acc. N.**
	Germany	SC-A1	S159	FJ820647
	Mexico	SC-A5	NY 8598	**Acc. N.**
	Morocco	SC-A1	MA-Fungi 5464	**Acc. N.**
	Portugal	SC-B1	MA-Fungi 26152	**Acc. N.**
	Romania	SC-A1	FCUG 1517	AF145572
	Russia	SC-A1	FCUG 2425	AF145571
	South Korea	SC-C1	KUC 8140	**Acc. N.**
	Spain	SC-B1	MA-Fungi 608	**Acc. N.**
	Spain	SC-B1	MA-Fungi 22499	**Acc. N.**
	Spain	SC-B1	MA-Fungi 12877	**Acc. N.**
	Spain	SC-B1	MA-Fungi 35643	**Acc. N.**
	Spain	SC-B1	MA-Fungi 75272	**Acc. N.**
	Spain	SC-B1	MA-Fungi 75310	**Acc. N.**
	Spain	SC-B1	MA-Fungi 12864	**Acc. N.**
	Spain	SC-A3	MA-Fungi 12880	**Acc. N.**
	Spain	SC-A3	MA-Fungi 12873	**Acc. N.**

Table 1 (cont.).

Label/ Voucher species name (Data Set 1)	Country	BGSR (Data Set 2)	Collection specimen	GenBank Accession n.º
Xylodon paradoxus (Schrad.) Chevall.	Spain	SC-A3	MA-Fungi 1063	**Acc. N.**
	Spain	SC-A3	MA-Fungi 5658	**Acc. N.**
	Spain	SC-A3	MA-Fungi 12772	**Acc. N.**
	Spain	SC-A3	MA-Fungi 12775	**Acc. N.**
	Spain	SC-A3	MA-Fungi 12771	**Acc. N.**
	Spain	SC-A1	MA-Fungi 5651	**Acc. N.**
	Spain	SC-A1	MA-Fungi 12794	**Acc. N.**
	Spain	SC-A1	MA-Fungi 46191	**Acc. N.**
	Spain	SC-A1	MA-Fungi 12857	**Acc. N.**
	Spain	SC-A1	MA-Fungi 12787	**Acc. N.**
	Spain	SC-A1	MA-Fungi 12844	**Acc. N.**
	Spain	SC-A1	MA-Fungi 12846	**Acc. N.**
	Spain	SC-C5	MA-Fungi 3269	**Acc. N.**
	Spain	SC-B1	MA-Fungi 12869	**Acc. N.**
	Spain	SC-B1	MA-Fungi 75244	**Acc. N.**
	Spain	SC-B1	MA-Fungi 12778	**Acc. N.**
	Spain	SC-B1	MA-Fungi 75130	**Acc. N.**
	Spain	SC-B1	MA-Fungi 22513	**Acc. N.**
	USA	SC-B2	HHB 719	**Acc. N.**
	USA	SC-B3	O-F 507276	**Acc. N.**
Xylodon raduloides Riebesehl & E. Langer				
	Argentina	SC-B4	FCUG 2492	AF145567
	Argentina	SC-B4	FCUG 2497	AF145566
	Argentina	SC-B4	ICMP 13832	AF145581
	Australia	SC-B5	ICMP 13833	AF145580
	Canada	SC-B5	FCUG 678	AF145564
	Chile	SC-B4	MES-2446	MH930325
	Chile	SC-B4	MA-Fungi 90703	**KY962841**
	Chile	SC-B4	MA-Fungi 90708	**KY962839**
	Chile	SC-B4	MA-Fungi 90705	**KY962835**
	Chile	SC-B4	MA-Fungi 90807	**Acc. N.**
	Chile	SC-B4	P.CH-4	KF562013
	Chile	SC-B4	MA-Fungi 90702	**KY962836**
	Chile	SC-B4	MA-Fungi 90706	**KY962838**
	Chile	SC-B4	MA-Fungi 90704	**KY962840**
	Denmark	SC-B1	FCUG-1972	AF145568
	France	SC-B1	MA-Fungi 70457	**Acc. N.**
	France	SC-B1	MA-Fungi 79442	**KY962834**
	France	SC-B1	MA-Fungi 79314	**KY962830**
	France	SC-B1	MA-Fungi 78658	**KY962828**
	France	SC-B5	MA-Fungi 74919	**KY962842**
	New Zealand	SC-B5	NZFS:4546	MH409968
	New Zealand	SC-B5	ICMP 13841	AF145579
	New Zealand	SC-B5	ICMP 13838	AF145578
	New Zealand	SC-B5	ICMP 13829	AF145577
	New Zealand	SC-B5	ICMP 13840	AF145576
	New Zealand	SC-B6	PDD 91616	GQ411525
	Rumania	SC-B1	FCUG 1055	AF145569

Table 1 (cont.).

Label/ Voucher species name (Data Set 1)	Country	BGSR (Data Set 2)	Collection specimen	GenBank Accession n.º
Xylodon raduloides Riebesehl & E. Langer	Russia	SC-B1	FCUG 2433	AF145570
	Spain	SC-B1	MA-Fungi 90709	**KY962844**
	Spain	SC-B1	MA-Fungi 75310	**Acc. N.**
	Spain	SC-B1	FCUG 2136	AF145565
	Turkey	SC-B1	FCUG 2239	AF141613
	USA	SC-B2	S.D. Russell MycoMap 8118	MK575271
	USA	SC-B2	DLL2011 142	KJ140643
	USA	SC-B2	DLL2009 049	JQ673187
	USA	SC-B2	DLL2009 087	JQ673189
	USA	SC-B2	DLL2009 082	JQ673188
	USA	SC-B7	UC2022947	KP814552

In addition to herbarium samples, a search at EMLB/GenBank/DDBJ and UNITE databases was performed in order to complete the molecular information available for each studied species. Geographic locations and species identification of each sequence were obtained from GenBank/UNITE vouchers.

Molecular methods and candidate species assignment through molecular barcoding

DNA extractions for *Xylodon* specimens from herbaria samples were performed. For DNA isolation, DNeasy™ Plant Mini Kit (Qiagen, Valencia, California, USA) was used, following the instructions of the manufacturers. Lysis buffer incubation was done overnight at 55 °C following (Whiting et al. 1997). In order to detect species candidates, a barcoding gap approach was utilized, using the Internal Transcribed Spacer (ITS) because this region is a universal barcode across fungi, able to detect genetic variability at the species level (Schoch et al. 2012). The ITS5/ITS4 (White 1990) primer combination was used to obtain DNA amplifications, of ITS1 and ITS2 regions plus 5.8S nrDNA. Amplifications were done using illustra™ PuReTaq™ Ready-To-Go™ PCR beads (GE Healthcare, Buckinghamshire, UK) as described in Winka et al. (1998), following thermal cycling conditions in Martín & Winka (2000). Negative controls lacking fungal DNA were run for each experiment to check for contamination of reagents. Results of amplifications were assayed from 5 µl aliquots by gel electrophoresis of 2 % Pronadisa D-1 Agarose (Lab. Conda, Spain). Amplified DNA fragments were first separated and purified from the agarose gel using the Wizard SV Gel and PCR Clean-Up System (Promega Corporation,

Madison, WI, USA) and sent to Macrogen Korea (Seoul, South Korea) for sequencing. Primers used for sequencing were those used for PCR amplifications. The ITS sequences generated were edited and assembled to obtain consensus using Geneious version 9.0.2, http://www.geneious.com (Kearse et al. 2012). The consensus sequences were lodged in the EMLB/GenBank/DDBJ databases with the accession numbers indicated in Table 1.

The matrix with all sequences obtained from herbarium samples was combined with sequences obtained from EMLB/GenBank/DDBJ databases (Table 1). MAFFT algorithm (Katoh & Standley 2013) was used to obtain sequence alignment for the ITS region; the automatic alignment was reviewed manually through Geneious version 9.0.2 and SeaView version 5.0.2.

A first general analysis was conducted with the whole DNA matrix in order to delimit big clades using pairwise distances under JC69 model and Neighbor-joining algorithm (Fig 2). After that, the Automatic Barcode Gap Discovery algorithm (ABGD, Puillandre et al. 2012) was applied to each delimited clade in order to obtain species candidates from the ITS region (barcoding gap species recognition, BGSR). ABGD algorithm uses the gap in the distribution of pairwise distances (i.e. the first statistically significant peak in the slope of ranked pairwise genetic distance values) of DNA barcode regions, assuming the divergence among organisms belonging to the same species is smaller than divergence among organisms from different species (Schoch et al. 2012). Consequently, specimens are grouped into species hypotheses or candidates that can be assessed later through the inclusion of molecular data from other regions or other sources of evidence (morphology, ecological preferences, biogeography, etc.).

The ABGD analysis was performed at http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html. The relative gap width was set to 0.5 to allow for the detection of closely-related taxa. The remaining parameters were set to default (Jukes-Cantor distance (JC69), Pmin=0.001, Pmax=0.100, Steps=10, Number of bins=20).

Species distribution modeling

Three kinds of predictor variables were included in distribution models, representing different factors that usually affect species distributions: abiotic, biotic and geographic variables (Soberón 2007). We used 19 environmental climatic (abiotic) layers from the Worldclim 2 database, https://www.worldclim.org/data/index.html (Fick & Hijmans 2017).

Table 2. Description of environmental predictors used in species distribution models.

Climatic	
	Isothermality – BIO3
	Temperature Seasonality – BIO4
	Mean Temperature of Wettest Quarter – BIO8
	Mean Temperature of Driest Quarter – BIO9
	Mean Temperature of Warmest Quarter – BIO10
	Mean Temperature of Coldest Quarter – BIO11
	Precipitation of Wettest Quarter – BIO16
	Precipitation of Driest Quarter – BIO17
	Precipitation of Warmest Quarter – BIO18
	Precipitation of Coldest Quarter – BIO19
Geographic	
	Latitude
	Sin (Longitude)
	Cos (Longitude)
Biotic	
	Tree cover

Variance Inflation Factor (VIF) scores were used to evaluate multicollinearity among abiotic predictors; the final set of climatic predictors used for modelling had VIF values lower than 2 (Zuur et al. 2010). After that, a total of 10 environmental climatic variables (Table 2) were included taking into account the importance of these factors reported by other studies (Wollan et al. 2008). Percentage of tree cover (biotic) from MODIS project (https://modis.gsfc.nasa.gov/data/dataprod/mod44.php) was also included as a predictor since *Xylodon* species are wood-decay fungi and depend on the existence of wood to grow and maintain their populations. Finally, to include pure geographic constrains that could affect species distributions limiting their dispersal or colonization capacity, latitude and longitude were included as predictor variables (Acevedo et al. 2012). Due to the circular character of longitude, the sine and cosine components were used instead (Pewsey et al. 2013).

Two data sets of presence records were built, depending on the species recognition criterion used to create them from the specimens analyzed: in Data Set 1, modeling groups were made based on taxonomic information of each specimen recorded from herbaria or sequence voucher name (Table 1); in Data Set 2, barcoding gap analyses results were used to re-group specimens following candidate species proposed by molecular barcoding analyses (BGSR). When the number of presences reported for a species candidate by BGSR was too low (less than 6), distribution model was not performed for such species

candidates due to the small size sample (Pearson et al. 2006; van Proosdij et al. 2016). The modeling approach was exactly the same for all arrangements in both data sets. We used the MaxEnt algorithm to conduct distribution models (Phillips et al. 2006, 2017). MaxEnt has been reported to perform well when only presence data (i.e. museum and herbaria data) is used, as in our case. Moreover, this algorithm has demonstrated acceptable accuracy for small size samples (Pearson et al. 2006). For each candidate species we ran 10 replicates with internal AUC (Area Under the Curve Receiver Operating Characteristic, Fielding & Bell 1997) validation (80% of presences for model calibration vs. 20% for model evaluation). Only linear, quadratic and product features were used to promote model interpretability (Merow et al. 2013) and cloglog output was selected (Phillips et al. 2017). In order to control the possible sample bias from our presence data sets, a layer of human footprint index was included as bias grid in MaxEnt, to represent those areas with more human accessibility as more probably sampled (Phillips et al. 2009; Elith et al. 2011). This index was obtained from "Last of the Wild Project", version 2. It consists of an overlay of a number of global data layers that represent the location of human population distribution, urban areas, roads, navigable rivers, and various agricultural land uses (http://sedac.ciesin.columbia.edu/data/collection/wildareas-v2). In order to evaluate the importance of each kind of predictor variable, percent contributions in model predictions were analyzed. To compare distributional range size between each species recognition criterion, distribution probability maps were transformed to presence/absence maps using equal test sensitivity and specificity threshold and the area occupied was calculated.

RESULTS

A total of 150 samples were considered in this study, of which 83 were newly sequenced (Table 1). Following genetic distance tree results (Fig 2), sequences were separated into 3 big clades, each one corresponding to one morphospecies: Clade A – *Xylodon paradoxus*, Clade B – *Xylodon raduloides* and Clade C – *Xylodon flaviporus*. Up to 19 species candidates were detected following BGSR: Data Set 2; Recursive Partition, prior intraspecific divergence P=0.002 (Table 1). The number of specimens assigned to each species candidate under BGSR varies from one to 37 (Table 1). In general, no clear correspondences were found among voucher names and species candidates detected under BGSR criterion, that is, no nested patterns were found (Table 1). For the specimens named *X. flaviporus*, eight different species candidates were detected under BGSR, while under

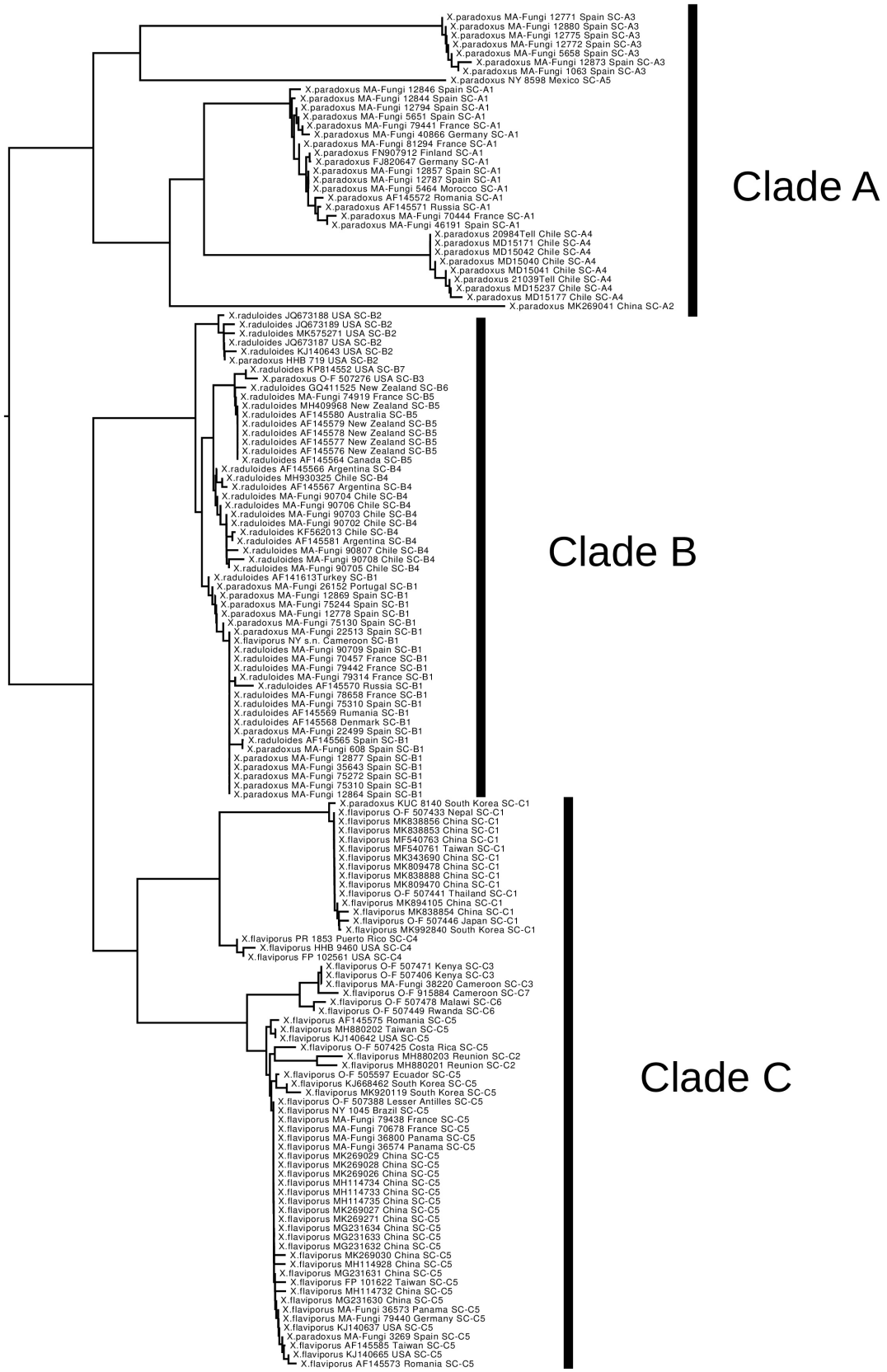

Fig 2. Neighbor-joining distance tree for the whole ITS nrDNA matrix used in this study. Big clades indicate sequences used in the three independent barcoding gap species recognition analyses conducted. Species names in herbarium and sequence vouchers and species candidates arrangements obtained from barcoding gap species recognition analyses are showed in tree tips.

the names *X. paradoxus* and *X. raduloides*, ten and six species candidates were detected through BGSR, respectively (Table 1).

Three distribution models were performed from Data Set 1 obtained following names in herbarium and sequence vouchers, one for each morphospecies. From the 19 species candidates detected under BGSR, the distribution models of only 9 species candidates were performed, those groups for which sample size was greater than 6 specimens (see Table 3, Fig 2).

The importance of each predictor in distribution models is shown in Table 3. Climatic variables obtained the highest percent contribution in models from names in herbarium and sequences vouchers (71.37% on average), followed by geographic predictors (21.64% on average) and finally by biotic variables (tree cover, 6.98% on average). However, for the models performed from species candidates detected by BGSR, the contribution of climatic variables was generally lower (41.68% on average; but see SC-A4, SC-B5 and SC-C5). Geographic predictors were the most important for five of the 9 species candidates under BGSR (53.74% of contribution on average), and tree cover had the least predictive value (6.98 percent contribution on average).

Table 3. Modeling results using both data sets: 1) Label and sequence voucher names and 2) Barcoding Gap Species Recognition (BGSR).

Data Set 1: Label and sequence voucher names					
	AUC	**% Occupied area**	**Predictors % contribution**		
			Climate	**Geography**	**Tree cover**
X. flaviporus (n = 62)	0.90	19 %	76.47	10.27	13.26
X. paradoxus (n = 50)	0.93	16 %	68.22	29.12	2.66
X. raduloides (n = 38)	0.91	14 %	69.43	25.54	5.03
Average			**71.37**	**21.64**	**6.98**

Data Set 2: Following Barcoding Gap Species Recognition (BGSR)					
	AUC	**% Occupied area**	**Predictors % contribution**		
Species Candidates			**Climate**	**Geography**	**Tree cover**
SC-A1 (n = 16)	0.95	9 %	26.33	72.95	0.72
SC-A3 (n = 7)	0.99	3 %	23.70	70.51	5.78
SC-A4 (n = 8)	0.99	< 1 %	60.28	39.47	0.24
SC-B1 (n = 25)	0.95	10 %	22.04	76.32	1.64
SC-B2 (n = 6)	0.99	< 1 %	30.52	64.61	4.86
SC-B4 (n = 12)	0.99	< 1 %	48.02	49.09	2.89
SC-B5 (n = 8)	0.92	14 %	56.08	32.60	11.32
SC-C1 (n = 15)	0.99	< 1 %	42.50	55.32	2.18
SC-C5 (n = 37)	0.90	18 %	65.63	22.81	11.55
Average			**41.68**	**53.74**	**4.57**

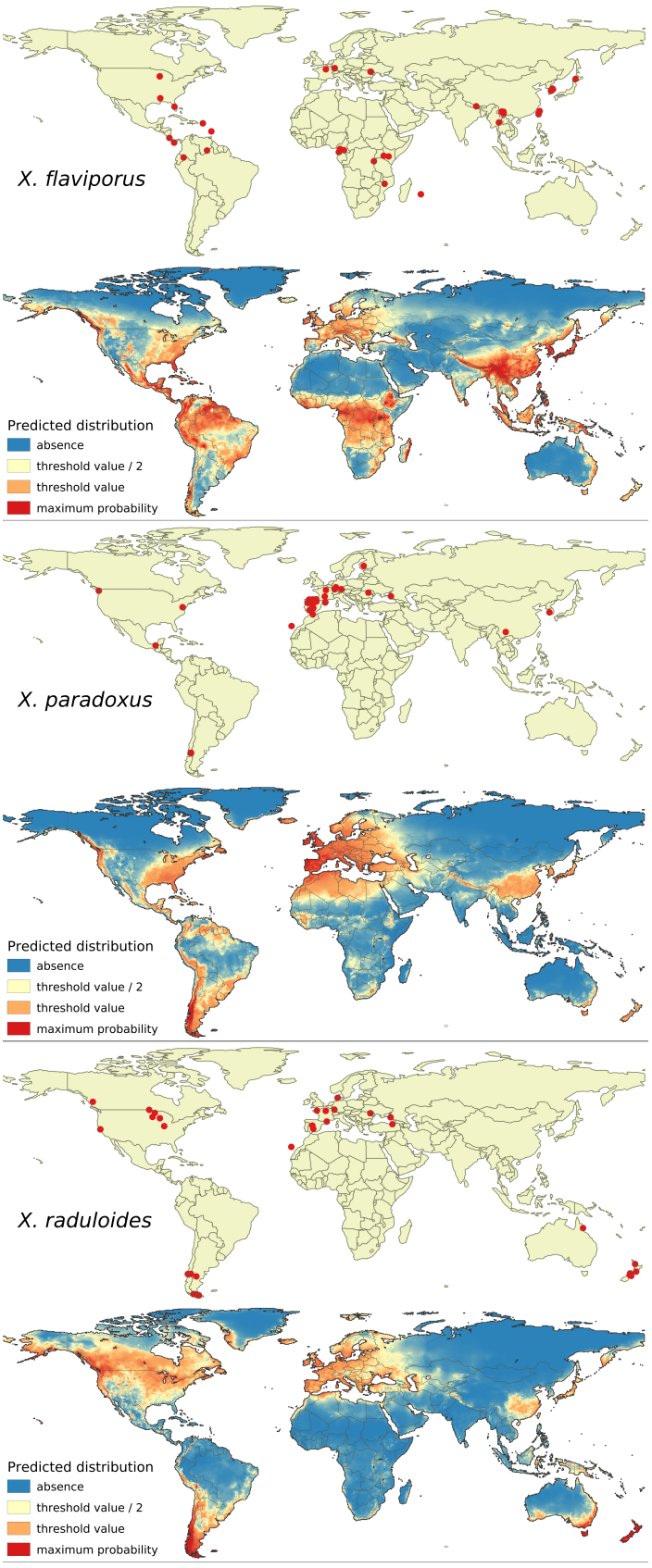

Fig 3. Presence records and distribution models for specimens arranged following labels and vouchers species names.

Distribution models built from voucher names showed worldwide distributions, and lacked biogeographic patterns (Fig 3). The extent of those distributions ranged from 14% to 19% of total worldwide emerged lands (Table 3).

In contrast, distribution models obtained for species candidates detected by BGSR showed in most cases local or restricted distributions (Fig 4). The distributions predicted from these models were in general smaller, with the exception of the species candidates SC-B5 and SC-C5. AUC values were always high, independent of the arrangement criterion used, with minimum and maximum between 0.90 and 0.99 (Table 3).

DISCUSSION

The development of new statistical tools to predict species distributions has promoted the use of presence-only databases such as natural history collections (Elith & Leathwick 2007). Herbaria or museum collections have been an important source of records to address biogeographical studies in macrofungi (Wollan et al. 2008). Nowadays, those techniques are commonly applied for a broad range of purposes, from assessing pest invasion risks to conservation management (Franklin 2013). They have also been used to evaluate the environmental factors that drive fungal species distributions (Wollan et al. 2008; Yuan et al. 2015), or to predict the potential distribution of ectomycorrhizal fungi under different climate change scenarios (Guo et al. 2017). However, the effects of taxonomic uncertainty have rarely been assessed in fungal distribution models (Elith et al. 2013). *Xylodon* is an adequate study case to understand those effects due to its high diversity and the lack of macroscopic diagnostic characters in many of its species (Riebesehl & Langer 2017).

Our results distinguished up to 19 species candidates under only three species names using molecular tools (Fig 2, Table 3). Although these species candidates are not all confirmed because a deeper study is needed (see Chapter 2 in this thesis for the case of Clade B – *Xylodon raduloides*), it draws a more realistic picture about the actual diversity in our presence records. This is a common pattern described in fungi, for which a single morphospecies hides many phylogenetic species that arise when molecular data is analyzed (Cai et al. 2014). Taxonomic issues are not fully solved in our analyses since only one DNA region was used, and multiple sources of evidence in an integrative framework is recommended to correctly define species boundaries (Dayrat 2005). However, it has been demonstrated that the ITS barcoding region generally performs well in fungal species

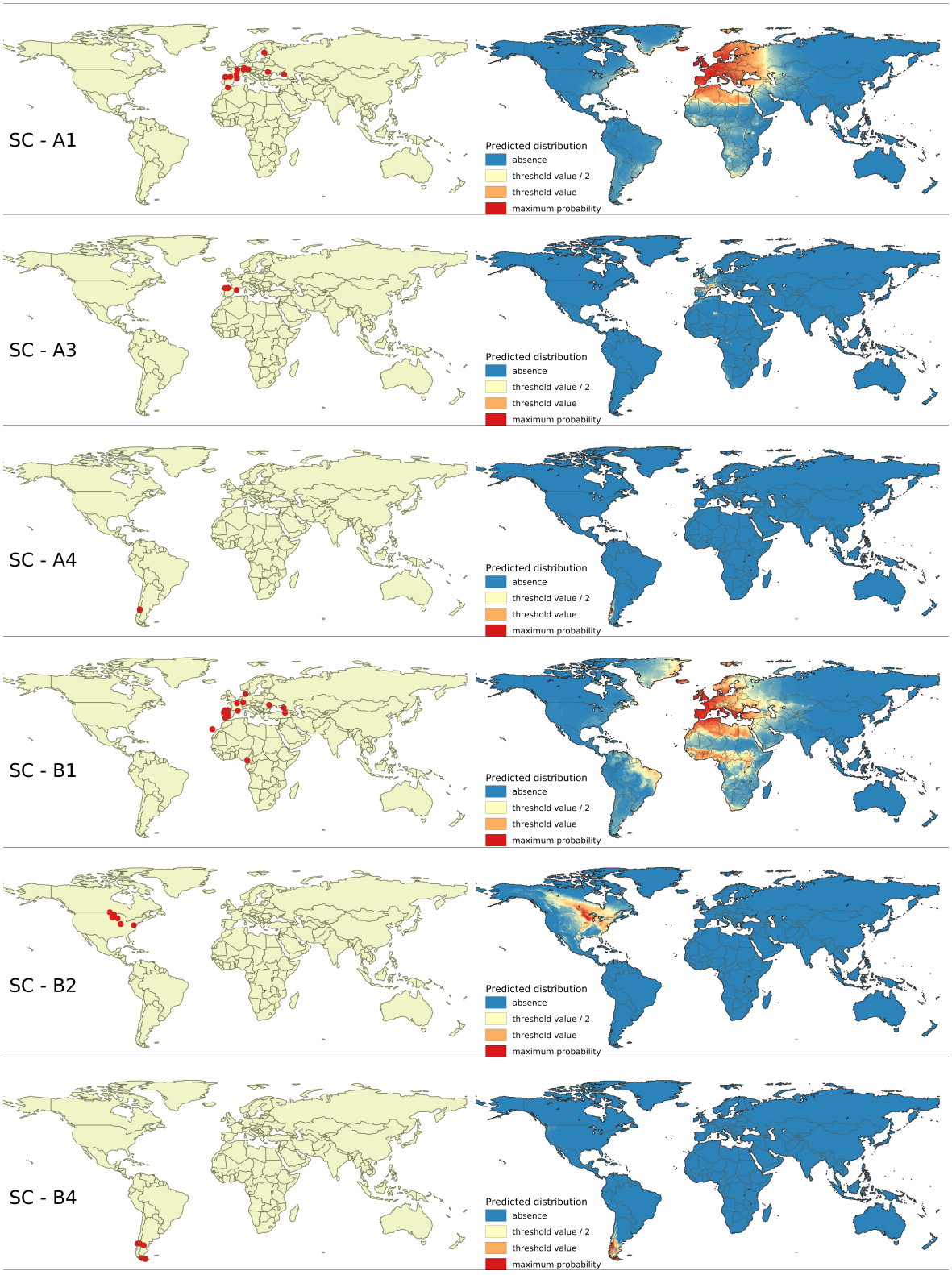

Fig 4. Presence records and distribution models for specimens arranged following ITS barcoding gap analyses (BGSR).

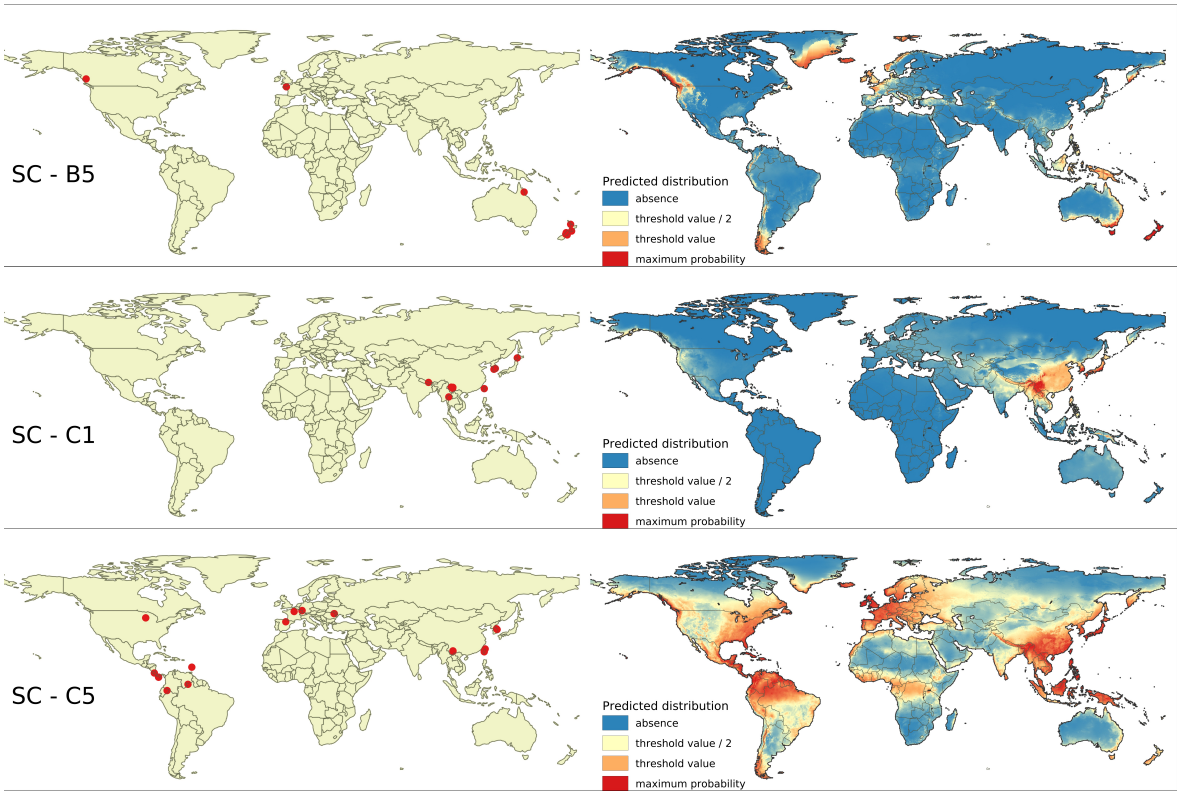

Fig 4 (cont.). Presence records and distribution models for specimens arranged following ITS barcoding gap analyses (BGSR).

delimitation (Schoch et al. 2012) and the barcoding gap approach is broadly used in fungal environmental studies (Tedersoo et al. 2014). Therefore, the species candidates delimited in this study are only a first step to understand the complexity in the available *Xylodon* data in different public collections.

Genetic analyses pointed towards two sources of misleading information in the studied material: first taxonomic uncertainty through cryptic speciation processes inside each morphospecies, since several subclades could be distinguished in the three big clades delimited (Fig 2). Second, an important amount of incorrect identifications especially between *X. paradoxus* and *X. raduloides*. These results could be expected due to the morphological similarities of these two species. In addition, *X. raduloides* was splitted from *X. paradoxus* in the late twentieth century (Hallenberg 1983), and therefore it is probable that several *X. raduloides* collections were still labeled under its old name.

Despite the relatively small sample size used in this study, our presence records described well the scope of the general distribution of the material available in natural history collections (Fig 1 and Fig 3). Predicted areas from models using label/voucher

information described cosmopolitan distributions for the three morphospecies. Predicted areas occupied up to 19 % of the world's emerged lands, and the three morphospecies can be found in Africa, America, Asia, Europe and Oceania. However, models derived from the molecular arrangement showed local or restricted distributions in most cases (except SC-B5 and SC-C5), with a biogeographic pattern (Fig 4). These reduced distributions support a more realistic picture of fungal diversity, since it has been demonstrated that genetic lineages remained at least partially isolated from each other in many fungi (Sato et al. 2012, Peay et al. 2010). It should be noted that the number of presence records for most of the candidate species is too small (Data Set 2) to affirm that predicted distributions reflect the actual species range i.e. species candidates SC-A3 and SC-A4 (Table 3). Thus, the lack of occurrences scattered over the actual species range could produce overfitted predictions and therefore distribution ranges can be underestimated. However, differences in distributions obtained between Data Set 1 and Data Set 2 are in accordance with similar patterns that have been reported in many Basidiomycota, for which there has been a transition from a few cosmopolitan species to numerous species with a regional distribution (Petersen & Hughes 1999). In addition, distant geographic origins in the lineages of each clade support the existence of different taxonomic entities under a single name.

Among the species candidates delimited by the BGSR approach, SC-B5 and SC-C5 maintained the worldwide distribution, with no biogeographic pattern supporting a genetic structure (Fig 4). In the case of SC-B5, this is due to two specific samples (one from France and another from Canada) and could be explained by human-mediated translocation, commonly reported for wood-decay fungi i.e. timber trade (Paulus et al. 2000; Fernández-López et al. 2019), since the rest of the samples are located in Australia–New Zealand. On the other hand, SC-C5 presents a much more complex pattern, with closely-related genetic samples distributed around the world. This pattern could be due to the inability of the barcoding approach to distinguish between these close-related species and therefore other sources of evidence or more DNA regions should be used to confirm this result (Balasundaram et al. 2015; Martín et al. 2018). Nevertheless, the hypothesis that the specimens arranged in this group are a single species with a worldwide distribution cannot be discarded.

The distribution predicted for species arranged following herbarium and sequence vouchers was mainly driven by climatic predictors rather than geographic or tree cover predictors (Table 3). It has been demonstrated that variables such as temperature or

precipitation play a central role in fungal distributions (Hao et al. 2020). However, for distribution modes derived from the genetic barcoding gap approach, although climatic factors remained important, they generally lost part of their predictive power in favor of geographic variables (Table 3). In our analyses, tree cover had less contribution than climatic or geographic factors. However, its contribution could be masked by climatic factors due to collinearity among predictors and therefore it should not be evaluated. Moreover, the resolution of cartographic layers could be too low to reflect the actual wood availability in small patches, where corticioid fungi can be present in isolated trees (Abrego et al. 2017).

It is important to highlight the inability of internal model validation in order to detect taxonomic uncertainty. Internal cross-validations are made by partitioning presence sample in training and test sets. Since test data has the same origin as training data, MaxEnt internal AUC is unable to detect wrong identifications in the occurrences. For this reason, AUC values for all models were always high (>0.90) independent of the Data Set used. In addition, it is known that the geographic coverage of a model influences AUC scores (Lobo et al. 2008). Since our study area is worldwide, AUC scores for our models are a misleading measure of model performance.

Our results demonstrate the important role that taxonomic uncertainty plays in the inferences obtained from species distribution models (Elith et al. 2013). Distribution patterns obtained from models based on names in herbarium and sequence vouchers supported the Baas Becking hypothesis "*Everything is everywhere, but environment selects*" in *Xylodon*. These unrealistic and overestimated distributions could similarly be assumed for other species that are involved in conservation programs or pest management plans, resulting in biological and economic loses (Bortolus 2008). In this context, preserved specimens in natural history collections offer the possibility to reevaluate occurrence data sets when taxonomic uncertainty may compromise the results obtained from species distribution models (Elith & Leathwick 2007).

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Appendix S1: Taxonomic status and synonymy of the selected morphospecies (www.indexfungorum.org, at 20 April 2020). Synonyms arranged chronologically; including their homotypic synonyms.

Xylodon flaviporus (Berk. & M.A. Curtis ex Cooke) Riebesehl & Langer, Mycol. Progr. 16(6): 646 (2017)

≡*Poria flavipora* Berk. & M.A. Curtis ex Cooke, Grevillea 15(no. 73): 25 (1886) ≡*Schizopora flavipora* (Berk. & M.A. Curtis ex Cooke) Ryvarden, Mycotaxon 23: 186 (1985) ≡*Hyphodontia flavipora* (Berk. & M.A. Curtis ex Cooke) Sheng H. Wu, Mycotaxon 76: 54 (2000) ≡*Kneiffiella flavipora* (Berk. & M.A. Curtis ex Cooke) Zmitr. & Malysheva , Nov. sist. Niz. Rast. 41: 146 (2008)

=*Poria confusa* Bres., Atti Imp. Regia Accad. Rovereto, ser. 3 3(1): 87 (1897) ≡*Aporpium confusum* (Bres.) Bondartsev, Trut. Grib Evrop. Chasti SSSR Kavkaza [Bracket Fungi Europ. U.S.S.R. Caucasus] (Moscow-Leningrad): 164 (1953)

=*Poria lignicola* Murrill, Mycologia 12(6): 307 (1920)

=*Poria jalapensis* Murrill, Mycologia 13(3): 177 (1921)

=*Polyporus trichiliae* Van der Byl, S. Afr. J. Sci. 18(3-4): 262 (1922) ≡*Schizopora trichiliae* (Van der Byl) Ryvarden, in Ryvarden & Johansen, Prelim. Polyp. Fl. E. Afr. (Oslo): 553 (1980)

=*Polystictus subiculoides* Lloyd, Mycol. Writ. 7(Letter 74): 1331 (1924) ≡*Schizopora subiculoides* (Lloyd) Ryvarden, Norw. J. Bot. 19(3-4): 236 (1972) ≡*Hyphodontia subiculoides* (Lloyd) Sheng H. Wu, Mycotaxon 76: 65 (2000)

=*Polyporus acaciae* Van der Byl, S. Afr. J. Sci. 22: 168 (1925)

=*Poria carneolutea* Rodway & Cleland [as 'carneo-lutea'], Pap. Proc. R. Soc. Tasm.: 18 (1930) ≡*Polyporus carneolutea* (Rodway & Cleland) Petr. [as 'carneo-lutea'], Petrak's Lists 6: 214 (1931) ≡*Schizopora carneolutea* (Rodway & Cleland) Kotl. & Pouzar, Česká Mykol. 33(1): 21 (1979)

=*Poria phellinoides* Pilát, Bull. Trimest. Soc. Mycol. Fr. 51(3-4): 383 (1936) ≡*Fibuloporia phellinoides* (Pilát) Bondartsev & Singer, Annls Mycol. 39(1): 49 (1941) ≡*Xylodon versiporus* var. *phellinoides* (Pilát) Domański, Acta Soc. Bot. Pol. 33(1): 168 (1964) ≡*Schizopora phellinoides* (Pilát) Domański, Acta Soc. Bot. Pol. 38(2): 255 (1969)

=*Xylodon versiporus* var. *microporus* Komarova, Botan. Mater. Otdela Sporovykh Rastenii, Bot. Inst. Akad. Nauk SSSR 12: 252 (1959)

=*Schizopora phellinoides* f. *tuberculata* Domański, Acta Soc. Bot. Pol. 38(2): 255 (1969)

~–*Poria curreyana* sensu Cunningham, fide Buchanan & Ryvarden (2000)

Xylodon paradoxus (Schrad.) Chevall., Fl. gén. env. Paris (Paris) 1: 274 (1826)

≡*Hydnum paradoxum* Schrad., Spicil. fl. germ. 1: 179 (1794) ≡*Sistotrema paradoxum* (Schrad.) Pers., Syn. meth. fung. (Göttingen) 1: 225 (1801) ≡*Lenzites paradoxus* (Schrad.) Pat., J. Micrographie 9: 23 (1885) ≡*Xylodon versiporus* f. *paradoxus* (Schrad.) Domański, Grzyby (Fungi): Podstawczaki (Basidiomycetes), Bezblaszkowe (Aphyllophorales), Zagwiowate I (Polyporaceae I), Szczecinkowate I (Mucronoporaceae I): 51 (1965) [1964] ≡*Schizopora paradoxa* (Schrad.) Donk, Persoonia 5(1): 76 (1967) ≡*Hyphodontia paradoxa* (Schrad.) Langer & Vesterh., in Knudsen & Hansen, Nordic Jl Bot. 16(2): 211 (1996) ≡*Kneiffiella paradoxa* (Schrad.) Zmitr. & Malysheva, Pyatnadtsataya Komi Respublikanskaya Molodezhnaya Nauchnaya Konferentsiya 2. Odinnadtsataya Molodezhnaya Nauchnaya Konferentsiya Instituta Biologii Komu NTs UrO RAN, 'Aktual'nye Problemy Biologii i Ekologii' (Materialy Dokladov), 19-23 Aprelya 2004 g. Syktyvkar, Respublika Komi, Rossiya (Syktyvkar): 103 (2004)

=*Hydnum obliquum* Schrad., Spicil. fl. germ. 1: 179 (1794) ≡*Sistotrema obliquum* (Schrad.) Alb. & Schwein., Consp. fung. (Leipzig): 263 (1805) ≡*Irpex obliquus* (Schrad.) Fr., Elench. fung. (Greifswald) 1: 147 (1828) ≡*Xylodon obliquus* (Schrad.) P. Karst., Acta Soc. Fauna Flora fenn. 2(no. 1): 31 (1881) [1881-1885] ≡*Coriolus obliquus* (Schrad.) Pat., Essai Tax. Hyménomyc. (Lons-le-Saunier): 94 (1900) ≡*Polyporus obliquus* (Schrad.) E.H.L. Krause, Mecklenburgs Basidiomyceten: 17 (1934) ≡*Poria versipora* f. *obliqua* (Schrad.) Kreisel, Phytopath. Grosspilze Deutschl.: 154 (1961) ≡*Xylodon versiporus* f. *obliquus* (Schrad.) Domański, Grzyby (Fungi): Podstawczaki (Basidiomycetes), Bezblaszkowe (Aphyllophorales), Zagwiowate I (Polyporaceae I), Szczecinkowate I (Mucronoporaceae I): 51 (1965) [1964]

=*Polyporus incertus* Pers., Mycologia Europaea 2: 106 (1825) ≡*Polyporus incertus* (Pers.) Sacc., Syll. fung. (Abellini) 6: 311 (1888) [nom.superfl.] ≡*Polyporus versiporus* subsp. *incertus* Pers., Mycol. eur. (Erlanga) 2: 106 (1825) ≡*Boletus incertus* (Pers.) Murrill, Mycologia 7(2): 78 (1920) ≡*Poria incerta* (Pers.) Murrill, Mycologia 12(2): 78 (1920)

=*Polyporus versiporus* Pers., Mycol. eur. (Erlanga) 2: 105 (1825) ≡*Poria versipora* (Pers.) Sacc., Syll. fung. (Abellini) 6: 311 (1888) ≡*Agaricus versiporus* (Pers.) E.H.L. Krause, Basidiomycetum Rostochiensium, Suppl. 4: 143 (1932) ≡*Xylodon versiporus* (Pers.) Bondartsev, Trut. Grib Evrop. Chasti SSSR Kavkaza [Bracket Fungi Europ. U.S.S.R. Caucasus] (Moscow-Leningrad): 128 (1953) ≡*Schizopora versipora* (Pers.) Teixeira, Revista Brasileira de Botânica 9(1): 44 (1986)

=*Polyporus versiporus* var. *angulatus* Pers., Mycol. eur. (Erlanga) 2: 105 (1825)

=*Polyporus versiporus* var. *farinosus* Pers., Mycol. eur. (Erlanga) 2: 106 (1825)

=*Polyporus versiporus* var. *deflexus* Pers., Mycol. eur. (Erlanga) 2: 106 (1825)

=*Polyporus versiporus* var. *immutatus* Pers., Mycol. eur. (Erlanga) 2: 105 (1825)

=*Polyporus versiporus* var. *lanuginosus* Pers., Mycol. eur. (Erlanga) 2: 106 (1825)

=*Polyporus versiporus* var. *sistotremoides* Pers., Mycol. eur. (Erlanga) 2: 105 (1825)

=*Irpex deformis* Schrad. ex Fr., Elench. fung. (Greifswald) 1: 147 (1828) ≡*Xylodon deformis* (Schrad. ex Fr.) P. Karst., Bidr. Känn. Finl. Nat. Folk 37: 66 (1882) ≡*Xylodon versiporus* f. *deformis* (Schrad. ex Fr.) Domański, Flora Polska, Grzyby (Mycota) 2: 51 (1965)

=*Daedalea mollis* var. *membranacea* Wormsk. ex Fr., Elench. fung. (Greifswald) 1: 72 (1828)

=*Daedalea mollis* Velen., České Houby 4-5: 690 (1922)

= *Irpex daedaleaeformis* Velen., České Houby 4-5: 743 (1922)

=*Poria laciniata* Velen. [as 'lacinatus'], České Houby 4-5: 638 (1922) ≡*Polyporus laciniatus* Velen., České Houby 4-5: 638 (1922) ≡*Schizopora laciniata* Velen., České Houby 4-5: 638 (1922)

=*Poria mucida* var. *radula* Bourdot & Galzin, Bull. Trimest. Soc. Mycol. Fr. 41(2): 237 (1925) ≡*Xylodon versiporus* var. *radula* (Bourdot & Galzin) Domański, Flora Polska, Grzyby (Mycota) 2: 52 (1965)

=*Irpex obliquus* f. *labyrinthiformis* Rodway & Cleland, Pap. Proc. R. Soc. Tasm.: 14 (1930) [1929]

=*Irpex obliquus* var. *argillaceocinnamomeus* Rodway & Cleland, Pap. Proc. R. Soc. Tasm.: 15 (1930) [1929]

=*Irpex decumbens* Rick, Egatea 17: 211 (1932)

=*Poria pseudoobducens* Pilát [as 'pseudobducens'], Atlas Champ. l'Europe, III, Polyporaceae (Praha) 1: 462 (1941) ≡*Poria pseudoobducens* Pilát, Acta Mus. Nat. Prag. 9: 107 (1953) [nom. superfl.] ≡*Oxyporus pseudoobducens* Pilát ex Bondartsev, Trut. Grib Evrop. Chasti SSSR Kavkaza [Bracket Fungi Europ. U.S.S.R. Caucasus] (Moscow-Leningrad): 551 (1953)

=*Irpex porosolamellatus* Rick, in Rambo (Ed.), Iheringia, Sér. Bot. 5: 187 (1959)

=*Poria albofulva* Rick, in Rambo (Ed.), Iheringia, Sér. Bot. 7: 282 (1960)

=*Poria membranicincta* var. *megalospora* Rick, in Rambo (Ed.), Iheringia, Sér. Bot. 7: 278 (1960)

=*Xylodon versiporus* var. *pseudoobducens* Pilát ex Domański, Acta Soc. Bot. Pol. 33(1): 167 (1964)

Xylodon raduloides Riebesehl & Langer, Mycol. Progr. 16(6): 649 (2017)

≡*Poria radula* Pers., Observ. mycol. (Lipsiae) 2: 14 (1800) ≡*Boletus radula* (Pers.) Pers., Syn. meth. fung. (Göttingen) 2: 547 (1801) ≡*Polyporus radula* (Pers.) Fr., Syst. mycol. (Lundae) 1: 383 (1821) ≡*Physisporus radula* (Pers.) Chevall., Fl. gén. env. Paris (Paris) 1: 262 (1826) ≡*Chaetoporus radula* (Pers.) Bondartsev & Singer, Annls mycol. 39(1): 51 (1941) ≡*Schizopora radula* (Pers.) Hallenb., Mycotaxon 18(2): 308 (1983) ≡*Hyphodontia radula* (Pers.) Langer & Vesterh., in Knudsen & Hansen, Nordic Jl Bot. 16(2): 212 (1996) ≡*Kneiffiella radula* (Pers.) Zmitr. & Malysheva, Pyatnadtsataya Komi Respublikanskaya Molodezhnaya Nauchnaya Konferentsiya 2. Odinnadtsataya Molodezhnaya Nauchnaya Konferentsiya Instituta Biologii Komu NTs UrO RAN, 'Aktual'nye Problemy Biologii i Ekologii' (Materialy Dokladov), 19-23 Aprelya 2004 g. Syktyvkar, Respublika Komi, Rossiya (Syktyvkar): 103 (2004)

=*Odontia cerasi* Pers., Observ. mycol. (Lipsiae) 2: 16 (1800) ≡*Sistotrema cerasi* (Pers.) Pers., Syn. meth. fung. (Göttingen) 2: 552 (1801) ≡*Hydnum cerasi* (Pers.) DC., Fl. franç., Edn 3 (Paris) 5/6: 36 (1815) ≡*Xylodon cerasi* (Pers.) Fr., Observ. mycol. (Havniae) 2: 267 (1818) ≡*Polyporus cerasi* (Pers.) Fr., Syst. mycol. (Lundae) 1: 382 (1821) ≡*Polyporus cerasi* (Pers.) Fr., Elench. fung. (Greifswald) 1: 148 (1828) ≡*Coriolus cerasi* (Pers.) Pat., Essai Tax. Hyménomyc. (Lons-le-Saunier): 94 (1900)

=*Hydnum flexuosum* Pers., Icones Pictae Rariorum fungorum 15: tab. 7 fig. 1 (1804) ≡*Sistotrema laevigatum* var. *flexuosum* (Pers.) Pers., Mycol. eur. (Erlanga) 2: 195 (1825)

=*Sistotrema leucoplaca* Pers., Mycol. eur. (Erlanga) 2: 196 (1825)

=*Sistotrema laevigatum* Pers., Mycol. eur. (Erlanga) 2: 195 (1825) ≡*Polyporus laevigatus* (Pers.) Duby, Bot. Gall., Edn 2 (Paris) 2: 785 (1830)

=*Sistotrema laevigatum* var. *amelanchieri* Pers., Mycol. eur. (Erlanga) 2: 196 (1825)

=*Sistotrema laevigatum* var. *funiculare* Pers., Mycol. eur. (Erlanga) 2: 196 (1825)

=*Sistotrema laevigatum* var. *heterodon* Pers., Mycol. eur. (Erlanga) 2: 196 (1825)

=*Radulum hydnans* Schwein., Trans. Am. phil. Soc., New Series 4(2): 164 (1832) ≡*Corticium hydnans* (Schwein.) Burt, Ann. Mo. bot. Gdn 13(3): 233 (1926)

=*Poria eyrei* Bres., Trans. Br. mycol. Soc. 3(4): 264 (1911)

=*Odontia macroverruca* H. Furuk., Bull. Govt. Forest Exp. Stn Meguro 261: 41 (1974)

–*Polyporus vaporarius* sensu Berkeley, fide Buchanan & Ryvarden (2000)

–*Poria vaporaria* sensu Colenso, fide Buchanan & Ryvarden (2000)

–*Poria versipora* sensu Lloyd, fide Buchanan & Ryvarden (2000)

Chapter 2

Addressing the diversity of *Xylodon raduloides* complex through integrative taxonomy

Javier Fernández-López, M. Teresa Telleria, Margarita Dueñas, Andrew Wilson, Mahajabeen Padamsee, Peter K. Buchanan, Gregory M. Mueller, María P. Martín

ABSTRACT

In this study, the taxonomic diversity of the *Xylodon raduloides* species complex (Hymenochaetales, Basidiomycota) is examined. Specimens were studied using an integrative taxonomic approach that includes molecular phylogenetic and morphological analyses, and environmental niche comparisons. Four different species were found inside the *Xylodon raduloides* complex, with a biogeographic distribution pattern bound by geographic regions: Europe, North America, Patagonia, and Australia–New Zealand. Molecular, morphological, and environmental evidences delimit two lineages within this complex: a Northern Hemisphere clade with longer basidiospores and wider ranges in temperature and precipitation tolerance, and a Southern Hemisphere clade with smaller and more spherical basidiospores, and an isothermal and more humid climate preference. The integrative taxonomic approach used in this study demonstrates congruence between data sets and shows how morphological and environmental characteristics contribute to the differentiation of fungal species complexes. By combining various sources of taxonomic information, three new species are described: *Xylodon laurentianus*, *X. novozelandicus*, and *X. patagonicus*.

INTRODUCTION

Corticioid fungi represent a polyphyletic group delimited by effused and resupinate basidiomes that usually grow on dead wood. One descriptor for these fungi is "paint on wood" which accurately characterizes their thin crust of reproductive structures, which are among the most elementary in Agaricomycetes. Although traditional classification grouped these fungi in a single family, molecular phylogenetic analyses have identified up to 50 different families in at least 11 orders (Larsson 2007).

Studying the biodiversity of corticioid fungi presents an opportunity to explore their phylogeny. First, despite their apparent macromorphological homogeneity, according to Mueller et al. (2007), there are more than 1800 described species, making them a highly diverse group. Second, they have colonized a broad range of environments around the world (Hallenberg 1991). For these reasons, corticioid fungi offer unique opportunities to study speciation and the geographic patterns that result from this process.

The idea that fungi are free from dispersal barriers had a long tradition, so global distributions were accepted as normal (Lumbsch et al. 2008). Cosmopolitanism or a high similarity index in corticioid fungi distribution patterns have been reported in many studies (Gilbertson 1980; Ghobad-Nejhad 2011). However, molecular studies have demonstrated distinct biogeographic patterns related to hidden biodiversity (Taylor et al. 2006; Knight & Goddard 2015). In this context, the "everything is everywhere" hypothesis (Baas Becking 1934) for all fungal groups has given way to the argument that geographic range inferred for a fungal species strongly depends on the nature of the characters used for its delimitation (Taylor et al. 2000).

Species concepts in fungi remain an important discussion topic (Taylor et al. 2000; Öpik et al. 2016). The same species concept is not always applicable across all fungal taxa due to the multiple evolutionary processes that can lead to fungal speciation (i.e. horizontal gene transfer, hybridization, etc.; Giraud et al. 2008). During the last two decades, molecular tools have transformed the study of fungal biodiversity. Among all the regions tested in Schoch et al. (2012), the nuclear ribosomal internal transcribed spacer DNA region (ITS, the fungal barcode) in most cases has the highest resolving power for discrimination among closely related species. Even so, taxonomic/systematic studies benefit by including other genetic regions (Balasundaram et al. 2015). Fungal species concepts have evolved through time (Cai et al. 2011) and mycologists have benefited from the development of genealogical concordance phylogenetic species recognition (GCPSR)

for describing fungal diversity (Taylor et al. 2000). Molecular data have provided a detailed view of previously hidden fungal diversity, enabling better use of traditional species recognition methods in morphology or mating compatibility to unmask this cryptic fungal diversity (Giraud et al. 2008). The implementation of GCPSR has revealed the presence of hidden diversity in several complexes of corticioid fungi where morphological species recognition approaches failed (e.g. *Serpula himantiodes*, Carlsen et al. 2011). However, with the increase of DNA regions used to estimate phylogenies, many researchers have argued the need to consider processes that could lead to discordance among gene phylogenies, that is, differences between gene-trees and species-trees (Edwards 2009; Heled & Drummond 2010). The use of different models, such as the coalescent theory (Kingman 1982) that allows gene tree heterogeneity, have been successfully applied for fungal species delimitation (e.g. *Hyphoderma paramacaronesicum*, Martín et al. 2018).

The search for evidence in addition to molecular phylogenetic data has emerged as a goal for species delimitation (Wiens 2007). Interest has increased in ecological traits as characters for species identification in many organisms (Rissler & Apodaca 2007). The combination of phylogenetics and niche modeling methodologies has proven useful in studying the mechanisms that shape biogeographic patterns (Raxworthy et al. 2007; Marske et al. 2012). With the development of GIS-based and cartographic approaches, the comparison of environmental niches has been proposed to study such evolutionary processes as sympatric speciation and niche conservatism (Warren et al. 2008; Broennimann et al. 2012; Ahmadzadeh et al. 2013). Due to the paucity of detectable macromorphological features in corticioid fungi, identification and comparison of environmental diagnostic traits could help in revealing their hidden diversity.

Xylodon (Pers.) Gray 1821 is a cosmopolitan white-rot fungus (Hymenochaetales, Basidiomycota), with an important role in ecosystem services due to their ability to alter wood structure and create habitat for other groups of organisms. As noted by Hibbett et al. (2014), it is one of the largest genera of wood-rotting fungi, with 162 current legitimate names (Robert et al. 2005; Robert et al. 2013). During the last ten years, at least six new *Xylodon* species have been described (Ariyawansa et al. 2015; Chen et al. 2018; Fernández-López et al. 2018; Viner et al. 2018), also 59 combinations made (Hjortstam & Ryvarden 2009; Riebesehl & Langer 2017; Chen et al. 2018) and two new names were proposed (Hjortstam & Ryvarden 2009; Riebesehl & Langer 2017).

Xylodon raduloides (Riebesehl & Langer 2017), previously known as *Schizopora*

radula (Hallenberg 1983), has been widely reported. It is widespread in Europe, and the Canary Islands (Hallenberg 1983; 1991; Langer 1994; Ryvarden & Gilbertson 1994; Melo et al. 2007; Ryvarden & Melo 2014), and it is also known from North America (Hallenberg 1983; Langer 1994; Zhou et al. 2016), South America (Langer 1994; Greslebin & Rajchenberg 2003; Gorjón & Hallenberg 2013; Martínez & Nakasone 2014), temperate Asia (Langer 1994; Hallenberg 1983), and Australasia (McKenzie et al. 2000; Paulus et al. 2000).

In a broad phylogenetic study of the genus *Schizopora* (now included in *Xylodon*), some degree of genetic isolation between populations of *X. raduloides* was detected, also supported by intercompatibility studies (Paulus et al. 2000). The worldwide geographic distribution of *X. raduloides*, along with available molecular data, suggested it could be a species complex with the true diversity reflecting biogeography. In this study, the diversity and biogeographic relationships in *X. raduloides* are addressed using an integrative taxonomic approach (Dayrat 2005). Our aim is to achieve a comprehensive understanding of the taxonomic diversity of the complex through the use of multiple sources of evidence (multi-locus species coalescent phylogeny, morphological characters, and environmental equivalence analysis).

MATERIALS AND METHODS

Taxon sampling and morphological studies

A total of 39 vouchers of *Xylodon* raduloides (Table 1) were obtained from four fungaria (CFMR, MA-Fungi, NY, and PDD), cultures of the Forest Products Laboratory (USDA), and ICMP culture collection (World Data Center for Microorganism 2011). Specimens from Huinay (Los Lagos Region, Chile) were collected during fieldwork in 2013/2014. Specimens encompassed a broad geographic range (Europe, North America, Patagonia, and Australia–New Zealand regions), in order to better understand the internal diversity and biogeography of the *X. raduloides* complex.

Table 1. Specimens and sequences included in this study. Data of country and basidiospore size are shown if available. New sequences obtained in this study in bold.

Species/specimens	Country	Basidiospore morphology			GenBank Accesion number			
		L	W	Q	ITS	LSU	*rpb2*	*tef-1α*
X. raduloides Riebesehl & E. Langer								
NY s.n.	Cameroon	5.75	3.87	1.49	**KY962843**	-	-	-
FCUG 1972	Denmark	-	-	-	AF145568	-	-	-
MA-Fungi 70457, 11074MD	France	5.33	3.37	1.58	**KY962827**	-	-	-
MA-Fungi 78658, 11851IS	France	5.38	3.15	1.7	**KY962828**	-	-	-
MA-Fungi 79314, 18336Tell.	France	5.03	3.3	1.52	**KY962830**	-	-	-
MA-Fungi 79442, 12028IS	France	5.15	3.32	1.55	**KY962834**	-	-	-
FCUG 1055	Romania	-	-	-	AF145569	-	-	-
FCUG 2136	Spain (Canary Islands)	-	-	-	AF145565	-	-	-
MA-Fungi 608	Spain	5.11	3.02	1.69	**KY962826**	-	-	-
MA-Fungi 12778, 2266MD	Spain	5.03	2.95	1.7	**KY962832**	-	-	-
MA-Fungi 12864, 755MD	Spain	5.07	3.13	1.62	**KY962820**	-	-	-
MA-Fungi 12877, 6996Tell.	Spain	5.25	2.75	1.91	**KY962821**	-	-	-
MA-Fungi 22499, 4719MD	Spain	5.4	3.35	1.61	**KY962822**	**KY962861**	-	-
MA-Fungi 22513, 4736MD	Spain	5.48	3.49	1.57	**KY962823**	**KY962862**	-	-
MA-Fungi 35643	Spain (Canary Islands)	6	4	1.5	**KY962831**	**KY962858**	**KY967054**	-
MA-Fungi 75130, GP2241	Spain	-	-	-	**KY962824**	**KY962863**	**KY967057**	**KY967079**
MA-Fungi 75244, GP2162	Spain	5.3	3.3	1.6	**KY962833**	-	-	-
MA-Fungi 75272, GP2253	Spain	6	4	1.5	**KY962829**	**KY962859**	**KY967053**	**KY967077**
MA-Fungi 75310, GP2291	Spain	5.16	3.05	1.69	**KY962825**	**KY962864**	**KY967055**	**KY967080**
MA-Fungi 90709, 002JFL	Spain	4.95	3.24	1.53	**KY962844**	**KY962860**	**KY967056**	**KY967078**
X. laurentianus sp. nov.								
CFMR, DLL2009-049	USA (Minnesota)	5.34	3.06	1.74	-	-	-	-
FPL (USDA), ex-culture DLL2009-049	USA (Minnesota)	-	-	-	JQ673187	**KY962866**	-	**KY967075**
CFMR, DLL2009-082	USA (Minnesota)	-	-	-	JQ673188	-	-	-
CFMR, DLL2009-087	USA (Minnesota)	-	-	-	JQ673189	-	-	-
CFMR, HHB-719, holotype	USA (Washington DC)	5.1	3.09	1.65	-	-	-	-
FPL (USDA), ex-culture HHB-719	USA (Washington DC)	-	-	-	**KY962845**	**KY962865**	-	**KY967076**
X. patagonicus sp. nov.								
ICMP 13832, ex-culture MR106	Argentina	-	-	-	AF145581	**KY962848**	**KY967058**	**KY967074**
MA-Fungi 90702, 14180M	Chile	5.26	3.51	1.5	**KY962836**	**KY962854**	**KY967062**	-
MA-Fungi 90703, 3567MPM	Chile	4.38	2.85	1.53	**KY962841**	-	-	-
MA-Fungi 90704, 3341MPM	Chile	4.58	3.18	1.44	**KY962840**	-	**KY967060**	-
MA-Fungi 90705, 14007MD	Chile	4.4	3.1	1.42	**KY962835**	-	**KY967063**	-
MA-Fungi 90706, 19705Tell.	Chile	4.56	3.17	1.44	**KY962838**	**KY962856**	**KY967064**	-
MA-Fungi 90707, 19684Tell., holotype	Chile	4.45	3.12	1.43	**KY962837**	**KY962855**	**KY967061**	-
MA-Fungi 90708, 3340MPM	Chile	4.32	2.9	1.49	**KY962839**	**KY962857**	**KY967059**	-
X. novozelandicus sp. nov.								
ICMP 13833, ex-culture PB 98/41	Australia	-	-	-	AF145580	**KY962853**	**KY967068**	**KY967073**
FCUG 678	Canada	-	-	-	AF145564	-	-	-
MA-Fungi 74919, 12836IS	France	5.2	3.15	1.65	**KY962842**	-	-	-
ICMP 13829, ex-culture PB 97/153	New Zealand	-	-	-	AF145577	**KY962850**	**KY967067**	**KY967071**
PDD 70716, Paulus 98/81	New Zealand	4.74	3.3	1.44	-	-	-	-
ICMP 13841, ex-culture Paulus 98/81	New Zealand	-	-	-	AF145579	**KY962852**	**KY967065**	**KY967072**

Table 1 (cont.).

Species/specimens	Country	Basidiospore morphology			GenBank Accesion number			
		L	W	Q	ITS	LSU	*rpb2*	*tef-1α*
X. novozelandicus sp. nov.								
PDD 70718, Paulus 98/20, holotype	New Zealand	3.77	2.8	1.35	-	-	-	-
ICMP 13838, ex-culture Paulus 98/20	New Zealand	-	-	-	AF145578	**KY962851**	-	**KY967069**
PDD 70720, Paulus 98/104	New Zealand	4.52	3.3	1.37	-	-	-	-
ICMP 13840, ex-culture Paulus 98/104	New Zealand	-	-	-	AF145576	**KY962849**	**KY967066**	**KY967070**
PDD 91616	New Zealand	5.34	3.48	1.53	GQ411525	-	-	-
X. flaviporus (outgroup)								
MA-Fungi 79440, 12094IS	Germany	-	-	-	**MH260071**	**MH260066**	**MH259319**	**MH758542**

Colours of dried basidiomata follow the ISCC-NBS Centroid Color Charts (Kelly & Judd 1976). Measurements and drawings were made from microscopic sections mounted in 3% aqueous KOH and Congo Red solution and examined at magnifications up to 1250× using an Olympus BX51 microscope. The length (L) and width (W) of 20 spores and 10 basidia were measured from each specimen. Mean values and length/width ratios (Q) for each spore were calculated (Table 1). Line drawings were made with a Leica DM2500 microscope with the aid of a drawing tube.

DNA extraction, amplification and sequencing

DNA isolation, amplification, purification and sequencing of four loci (ITS nrDNA, LSU nrDNA, tef-1α, rpb2) were performed following Martín et al. (2018). The raw sequences generated were edited, and contigs were assembled using Geneious version 9.0.2 (Kearse et al. 2012). Consensus sequences were accessioned in the EMBL/GenBank/DDBJ databases, and accession numbers are presented in Table 1.

Evaluation of EMBL/GenBank/DDBJ databases for *X. raduloides* sequence data was performed to maximize the molecular information available for this taxon. One sequence from *Xylodon flaviporus* (Riebesehl & Langer 2017) per marker was added to each dataset as an outgroup in phylogenetic analyses. MAFFT (Katoh & Standley 2013) was used to obtain sequence alignment for each region, with additional evaluation and edits of alignment performed using Geneious. A fifth alignment was performed for combined ITS + LSU sequence data. For this dataset, samples that lacked the LSU sequence were given a string of '?' to represent missing data.

Phylogenetic analyses

Combined ITS + LSU phylogenetic tree estimation was performed using Bayesian inference (BI) implemented in BEAST v2.4.3 (Drummond & Rambaut 2007; Bouckaert et al. 2014). Site model partition was defined using BEAUti v2.4.3 interface for ITS and LSU separately. HKY+G substitution model was selected for both, as the closest available in BEAST from the results obtained in jModelTest2 (Darriba et al. 2012) following Bayesian information criterion (BIC). We used relative timing with an uncorrelated lognormal relaxed clock by calibrating the tree with a value of 1 in the root for the *X. raduloides* clade (Drummond et al. 2006). Coalescent (constant sites) model was used as tree prior. Two MCMC runs were specified for 50 million generations, sampling every 5000th generation. Tree and log files were combined in Logcombiner v.1.7 and results were visualized in Tracer v.1.6 (Rambaut et al. 2018), to evaluate whether the effective sample size (ESS) values were above 200. The resulting trees were summarized in a maximum clade credibility tree by TreeAnnotator v.1.7. with a burn-in of 5,000. The same procedure was used to separately estimate phylogenetic trees for each region (ITS and LSU).

A multi-locus species coalescent approach was used to test alternative species delimitation hypotheses (Grummer et al. 2014). To accomplish this, a competing species delimitation model was used (Fig 1). We explored a priori assignment of individuals to lineages following three hypotheses based on different sources of information. The first hypothesis (hypothesis-A) addressed traditional taxonomic classification, including all *X. raduloides* specimens as a single species, separated from *X. flaviporus* as outgroup. In the second hypothesis (hypothesis-B) geospatial characters were used by grouping specimens according to a North-South distribution, including all specimens from the Northern Hemisphere as a first species and assigning all specimens from the Southern Hemisphere as a second species. Finally, a DNA barcoding species delimitation obtained from the ITS tree was addressed in the third hypothesis (hypothesis-C), assigning each specimen to the species defined by the ITS tree.

We estimated species trees for each model using the coalescent-based inference program *BEAST with the four amplified DNA regions (ITS, LSU, tef-1α and rpb2). This method co-estimates gene and species trees from sequence data taking into account evolutionary processes that could generate species-tree/gene-tree discordance, such as incomplete lineage sorting (Mallo & Posada 2016). Substitution and clock models for each region were selected in the same procedure as for the ITS + LSU tree. Constant population

function (population mean=1) was used to model the species tree population size. A coalescent constant population prior was used to build the species tree. Tree and log files were managed in the same way as for the ITS + LSU tree (ESS values above 200; burn-in =5000). In order to visualize the species tree and concordance between the four DNA regions, the Densitree v2.01 package included in BEAST v2.4.3 was used (Bouckaert 2010).

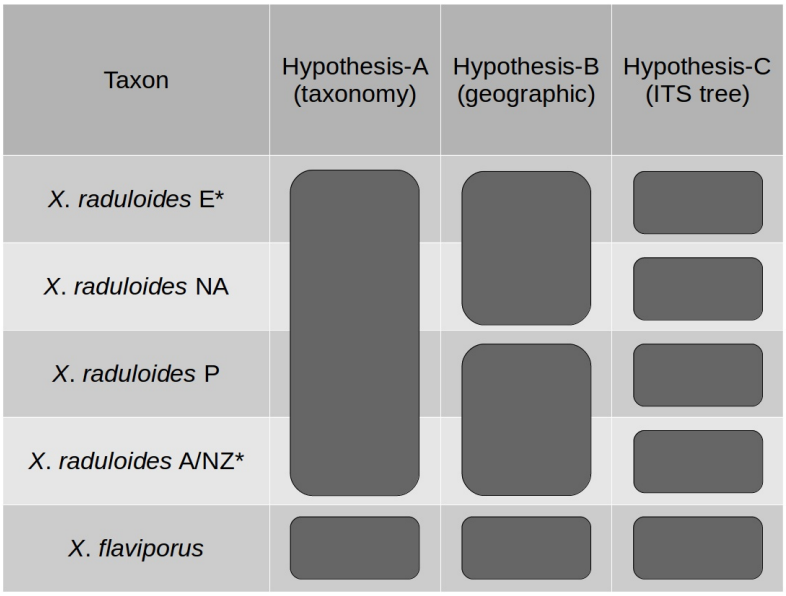

Fig 1. Hypothesized specimen assignment to each species hypothesis-model tested in this study. Species delimitation models are shown as columns, while lineages are shown as rows. Letters indicate geographic regions: E=Europe; NA=North America; P=Patagonia; A/NZ=Australia–New Zealand. (*) In Model-C, European taxon includes one sample from Cameroon (NY s.n.) and Australia–New Zealand taxa includes one specimen each from France (MA-Fungi 74919) and Canada (FCUG 678).

To assess the suitability of each species delimitation hypothesis proposed, Bayes factor delimitation (BFD) was performed following the framework of Grummer et al. (2014). Marginal likelihood for each hypothesis (MLEs), measured as log likelihoods, are calculated from the Bayesian posterior distributions through stepping-stone analyses using Path Sampler Analyser (BEAST model-selection package version 1.0.2; default parameters: alpha=0.3, steps=24, chain length=100000 and burn-in=50%). Bayes factors are calculated as two times the difference in MLE between the best-fitting and alternative hypothesis (2lnBf). Hypothesis-C was used as the standard to compare against the other two hypotheses (hypothesis-C vs hypothesis-A and hypothesis-C vs hypothesis-B). Values of 2lnBf between 0-2 are interpreted as no significant differences in support for the two

hypotheses. Values over 10 means decisive support in favor of the best-fitting hypothesis over its alternative (Kass & Raftery 1995).

Statistical tests of morphological characters

Basidiospore morphology was analyzed since sexual structures are valuable for species differentiation in fungi. One-way ANOVA tests were performed to assess the significance of differences in spore morphology between clades identified from multi-locus species coalescent analyses. Differences in spore morphology were evaluated using a Tukey HSD post-hoc test. Exploratory plots (i.e. residuals vs fitted values, normal Q-Q plots and residuals vs leverage) were used to detect and remove outliers from the morphological dataset. Two specimens (MA-Fungi 90702 and MA-Fungi 74919) from the total of 30 in the morphological dataset were removed after outlier identification.

Environmental niche equivalence analyses

We examined environmental characteristics to assess the degree of niche equivalency between species delimited with multi-locus species coalescent analyses. For each of the studied specimens (out-group excluded), geographical location (longitude and latitude) was obtained from herbarium labels or collection information. A set of 19 bioclimatic variables related to temperature and precipitation were obtained from the WorldClim2 cartographic dataset (Fick & Hijmans 2017, Table 2).

To characterize the bioclimatic niche for each species, a total of 10,000 random points was generated over the entire study area (worldwide) and their bioclimatic features were extracted from the 19 variables. This dataset, in addition to bioclimatic values for specimen locations, was used to calibrate a Principal Components Analysis (PCA) that represents the environmental space. Then, a kernel density function was applied to obtain the smoothed density of species occurrences in this environmental space. Further niche overlap analyses were performed using these kernel distributions rather than actual species occurrences (Broennimann et al. 2012).

In order to assess whether environmental niche can be used as a diagnostic character to distinguish between species of the *X. raduloides* complex, equivalence tests were performed using Schoener's D index (Schoener 1970). This index is a metric of ecological similarity that ranges from 0 (no niche overlap) to 1 (complete overlap).

Equivalence tests were conducted by comparing the environmental overlap values (D) of pairs of *Xylodon* species to a null distribution of 100 randomized overlap values. We determined non-equivalence of environmental niches if the niche overlap value of the two species being compared differed significantly from the overlap values from the null distribution. All analyses were performed in the R platform (R Development Core Team, 2014) using "ecospat" R package v1.0 (Di Cola et al. 2017).

Table 2. Description of bioclimatic variables used in environmental niche modeling from WorldClim2.

Name	Variable
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (maxtemp-mintemp))
BIO3	Isothermality (BIO2/BIO7)(*100)
BIO4	Temperature Seasonality (standard deviation * 100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

RESULTS

DNA extraction, amplification and sequencing

A total of 77 sequences were generated in this study: 27 sequences for ITS region, 20 for LSU, 17 for rpb2 and 13 for tef-1α (Table 1). The maximum lengths of sequences were 618 for ITS, 1347 for LSU, 884 for rpb2 and 748 for tef-1α. The final alignments, including sequences retrieved from the EMBL/GenBank/DDBJ databases contained 41 ITS sequences for a dataset length of 502 characters, 20 LSU sequences with 772 characters, 17 rpb2 sequences with 646 characters and 13 tef-1α sequences with 613 characters. No *X. raduloides* sequences were available for rpb2 and tef-1α regions from the EMBL/GenBank/DDBJ databases.

Phylogenetic analysis

Results of the phylogenetic analysis of ITS, LSU, and ITS + LSU alignments are summarized in Fig 2. All effective sample sizes were higher than 200 for all parameters. Bayesian inference analyses suggest the division of *Xylodon raduloides* complex into four well-supported monophyletic clades (posterior probabilities (PP) ≥ 0.96 for all clades in the ITS tree), each restricted to their geographical distribution: Europe, North America, Patagonia, and Australia–New Zealand (Fig 2). The only exceptions to this strong geographic pattern were two specimens in the Australia–New Zealand molecular clade that came from Europe and North America, while one specimen from Africa was resolved within the European clade.

Marginal likelihoods from multi-locus species coalescent analyses for each hypothesis and Bayes factors for hypothesis comparisons are shown in Table 3. The species hypothesis provided by the ITS barcoding approach (hypothesis-C) was the most probable scenario following Bayes factor scores (hypothesis-C vs hypothesis-A 2lnBf=78.88; hypothesis-C vs hypothesis-B 2lnBf=22.24), obtaining a Bayes factor > 10 for the second more probable scenario ("decisive" support for hypothesis-C against hypothesis-B). The species tree obtained from the multi-locus species coalescent approach for hypothesis-C defined four species divided into two well-supported clades (PP > 0.98; Fig 3). The first clade grouped the two Holarctic species (European and North American species) and the second clade included Antarctic-Australian species (Patagonian and Australian–New Zealand species). The MCMC sample of gene trees obtained from *BEAST analyses for hypothesis-C was visualized by Densitree v2.01 and showed a high level of genealogical concordance with single DNA region trees (Fig 3).

Statistical tests of morphological characters

ANOVA on basidiospore width, length and length/width ratio was conducted on 15 European specimens; one sample from Cameroon (NY s.n.); two North American specimens; six Patagonian specimens; and four Australia–New Zealand specimens (Fig 4). The analysis did not detect any difference in spore width between species ($F(3, 24)=1.53$, P-value=0.23). However, differences were detected in spore length and length/width ratios (Q) between Northern (Europe and North America) and Southern (Patagonia and Australia-New Zealand) hemisphere samples ($F(3, 24)=11.52$, P-value < 0.05 and $F(3, 24)=7.96$, P-value < 0.05 respectively; Fig 4).

Table 3. Marginal likelihood estimates from each species tree hypothesis and Bayes factors (2lnBf) for hypotheses comparisons.

MLE		2lnBf	
Hypothesis – A	-5471.95	Hypothesis – C vs Hypothesis – A	78.88
Hypothesis – B	-5442.63	Hypothesis – C vs Hypothesis – B	20.24
Hypothesis – C	-5432.51		

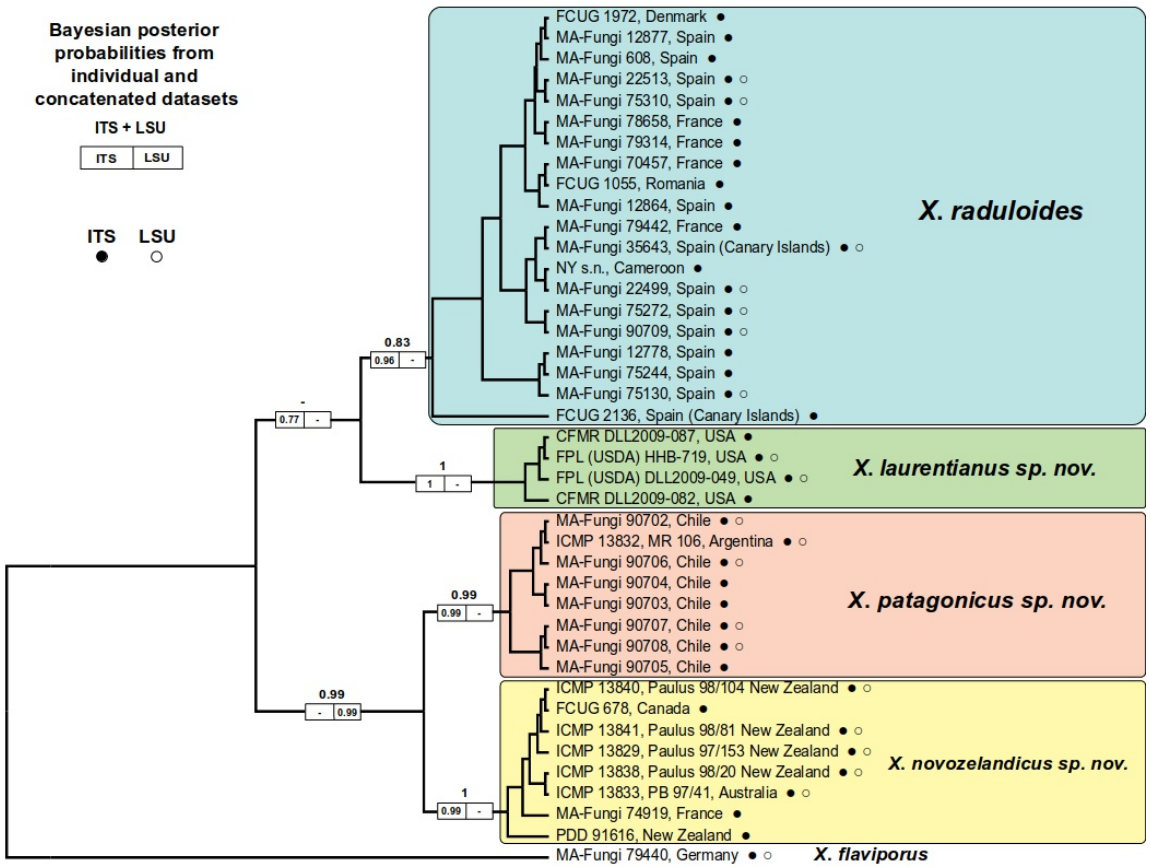

Fig 2. Topology of ITS + LSU tree obtained by Bayesian inference using BEAST. Bayesian posterior probabilities for the combined ITS and LSU dataset are indicated in the larger number above the boxes. Individual gene posterior probabilities for ITS and LSU regions are indicated in the left and right boxes on a branch, respectively. Filled and empty circles indicate whether ITS or LSU sequence of a sample was used in the analysis.

In the post-hoc Tukey HSD tests significant differences were found for spore length/width ratios between inter-hemisphere comparisons: Europe and Patagonia; Europe and Australia-New Zealand; North America and Patagonia; and North America and Australia–New Zealand (P-values < 0.05). However, the Tukey HSD test did not show

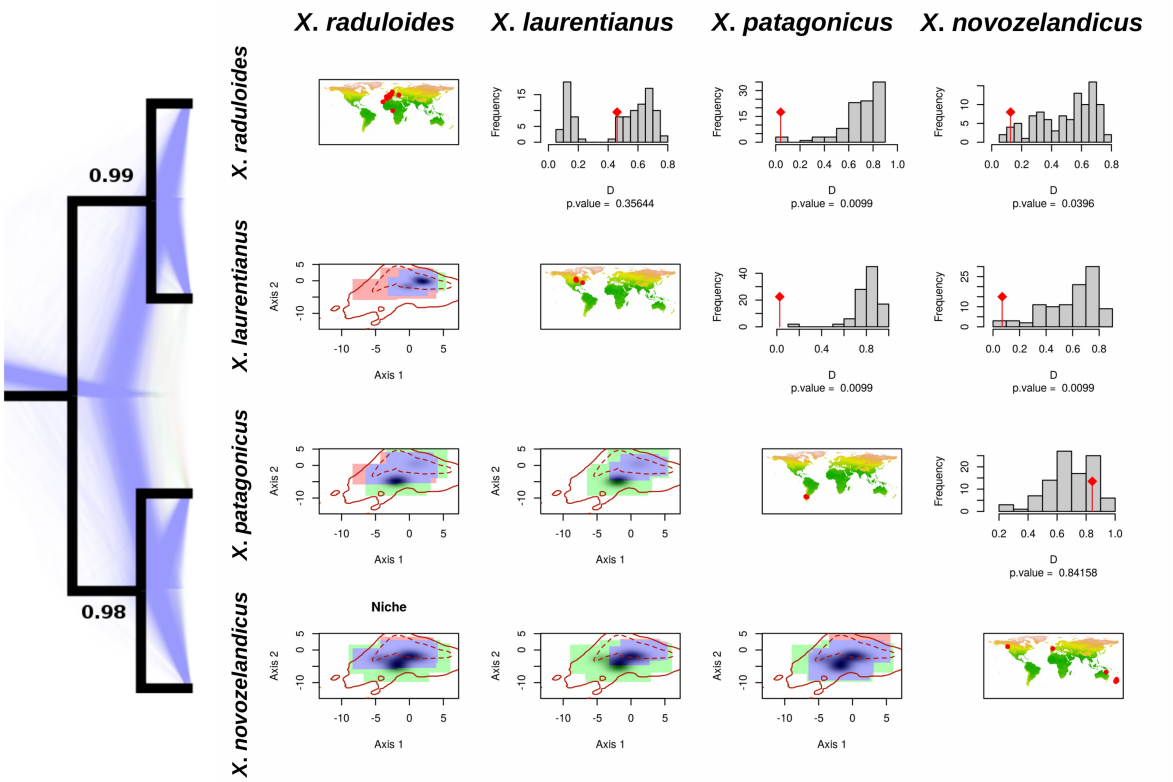

Fig 3. Species tree obtained from *BEAST applying multi-locus species coalescent approach for the four DNA regions used in the analyses (ITS, LSU, rpb2 and tef-1α) and results from niche overlap analyses. Tree topology represents Model-C obtained from the ITS analyses. Posterior probabilities are shown as supports for each node. Blue background trees represent bootstrap gene tree topologies obtained from *BEAST. Diagonal maps show locations of the specimens. The upper right triangle of the matrix represents the results from niche overlap analyses. P-values of the equivalence test are shown above each histogram of randomization procedures. The bottom left triangle of the matrix shows species niches resulting from PCA analysis. The blue areas show overlapping environmental niches, while green and red represent niche ranges for row and column species, respectively. Shaded areas indicate kernel distributions for row species. Solid and dotted lines represent environmental availability for the whole study area.

significant differences in spore length in the inter-hemisphere comparison between North America and Australia–New Zealand (P-value=0.19).

Environmental niche equivalency analyses

The PCA performed with the 19 bioclimatic variables accumulated 76.71% of the variance in the first two axes (Axis 1: 50.32%; Axis 2: 26.39%; Fig 3). For niche comparisons, two axes rather than one were selected to obtain a more complete view of niche dimensions,

since the first axis represented only 50% of environmental variability. Axis 1 described mainly a gradient between regions with high temperature seasonality (BIO4) and high temperature annual range (BIO7) (positive values in axis 1) and areas with a high

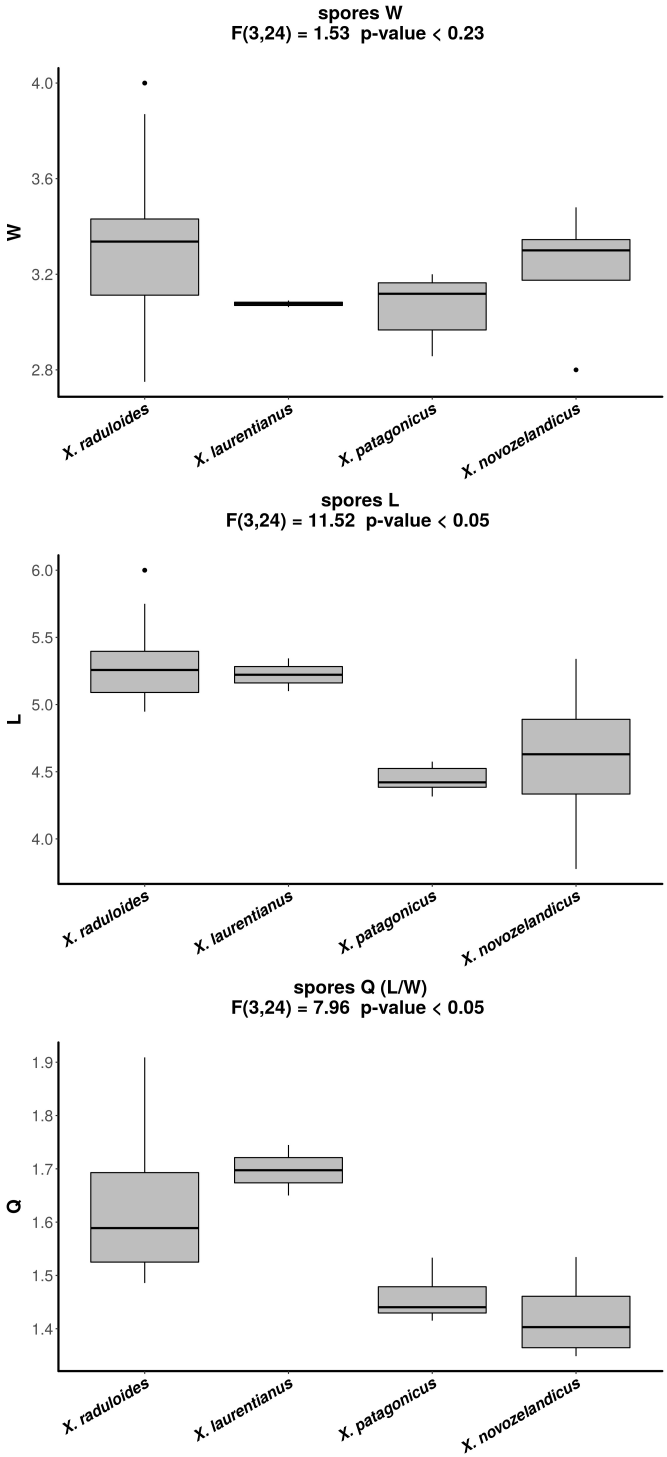

Fig 4. ANOVA results for basidiospore morphology. Bar plots of Xylodon species basidiospore width (W), length (L) and length/width ratios (Q).

isothermality (BIO3) (negative values in axis 1). The environmental pattern of Axis 2 was less clear with areas of high temperature diurnal range (BIO2) and high precipitation seasonality (BIO15) on one side (positive values in axis 2) and regions with high precipitation during dry seasons (BIO14, BIO17) on the other side (negative values in axis 2). Kernel densities were then built for each clade from the coordinates (PC scores) of group occurrences in this two-dimensional environmental space created by the two first axes of the principal components analyses.

In general, environmental space occupied by North American and European occurrence kernels were placed in higher values for both axis 1 and axis 2 in the PCA (top-right) than Patagonian and Australia–New Zealand kernels (bottom-left, Fig 3). That pattern describes a more seasonal niche for Northern Hemisphere species, while Southern Hemisphere species showed preferences for isothermal and humid areas.

Niche equivalence could not be rejected between North American and European species (P-value=0.35, Fig 3). A similar pattern was found for Patagonian and Australia–New Zealand niches, where the niche equivalence hypothesis could not be rejected between these two species (P-value=0.84, Fig 3). However, when inter-hemisphere environmental niches were compared, significant differences were observed for all species (niche equivalence was rejected; P-values < 0.05, Fig 3).

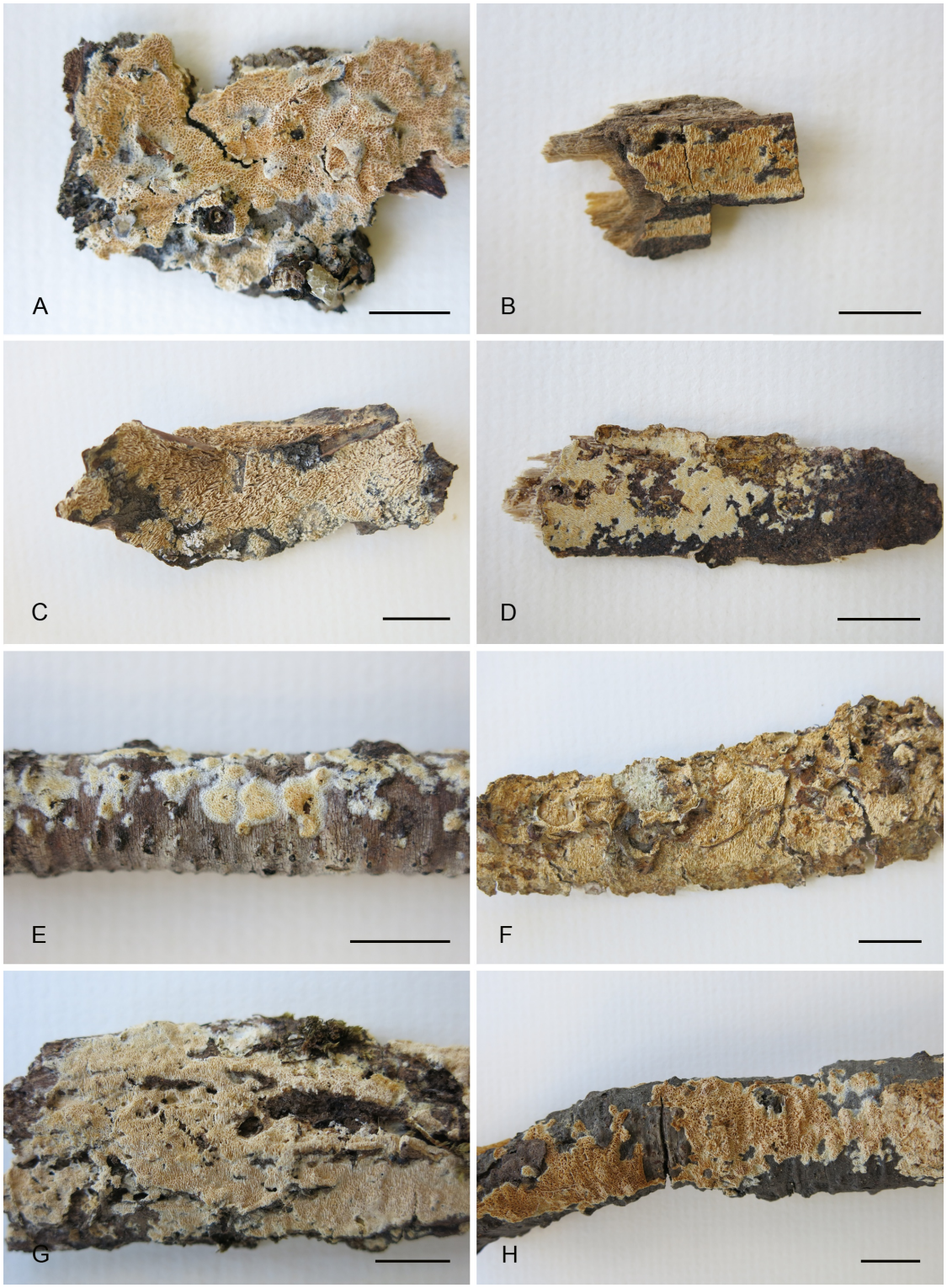

Fig 5. Basidioma of *Xylodon species*. A. *Xylodon raduloides* (755MD, MA-Fungi 12864). B–C. *Xylodon laurentianus* (B. HHB-719, CFMR, holotype; C. DLL2009-049, CFMR). D–F. *Xylodon patagonicus* (D. 19684Tell., MA-Fungi 90707, holotype; E. 14180MD, MA-Fungi 90702, young specimen; F. 19705Tell., MA-Fungi 90706, old specimen). G–H. *Xylodon novozelandicus* (G. Paulus 98/20, PDD 70718, holotype; H. Paulus 98/104, PDD 70720). Bars=1 mm.

Based on a combination of taxonomic information, three new species are described here: *Xylodon laurentianus*, *X. novozelandicus*, and *X. patagonicus*.

TAXONOMY

Xylodon raduloides Riebesehl & Langer, Mycol. Progr. 16: 649 (2017).

Replaced name: *Poria radula* Pers., Observ. Mycol. 2: 14 (1800).
Synonyms: *Polyporus radula* (Pers.) Fr., Syst. Mycol. 1: 383 (1821); nom. sanct.
Schizopora radula (Pers.) Hallenb., Mycotaxon 18: 308 (1983).
Hyphodontia radula (Pers.) Langer & Vesterh., Nordic J. Bot. 16: 212 (1996).
Kneiffiella radula (Pers.) Zmitr. & Malysheva, Pyatnadts. Respubl. Molod. Nauchn. Konf.: 103 (2004).
Non *Xylodon radula* (Fr.) Tura et al., Biodiv. Heterobasid. non-gilled Hymen. Israel: 219 (2011); based on *Hydnum radula* Fr., Obs. Mycol. 2: 271 (1818); nom. sanct.

Type: [locality unknown, substrate unknown], "*Poria radula*" [Persoon's hand] (L0117159 [Herb. Ludgd. Bat. 910.277-305] – neotype designated by Donk 1967: 106, as "type").

Description: Basidioma resupinate, effuse, adnate; hymenophore poroid, 1–4 pores/mm, regular to angular, dissepiments dentate in old specimens, on vertical substrata irregularly irpicoid with flattened teeth, yellow-white to orange-yellow (92. y White – 71. m. OY); margin clearly differentiated, paler (Fig 5A). Hyphal system monomitic; generative hyphae hyaline, thin to thick-walled, sparsely branched, with clamps, 3–5 µm wide; subicular hyphae loosely interwoven, parallel to substratum (Fig 6A); subhymenial hyphae, perpendicular to the substratum (Fig 6B). Cystidia or rather cystidial elements present: (1) capitate, subcylindrical, fusiform or moniliform cystidia arise from the hymenium (Fig 6C), sometimes encrusted or with an apical bladder, thin-walled, with basal clamp, 17–26 × 3–5 µm; (2) capitate hyphae arise from the subiculum (Fig 6D), sometimes with a thin-walled apical bladder, thin to thick-walled, with basal clamp, 30–40 × 3–4.5 µm, apex to 9 µm diam; and (3) tubular hyphae or "skeletocystidia" arise from the subiculum (Fig 6E), with very thick walls narrowing to the apex, with basal clamp, 120–150 × 3–5 µm. Basidia cylindrical to suburniform, (15–)18–21 × 4–5 µm, four sterigmata, with basal clamp (Fig 6C). Basidiospores ellipsoidal, (4.5–)5–5.5(–7) ×

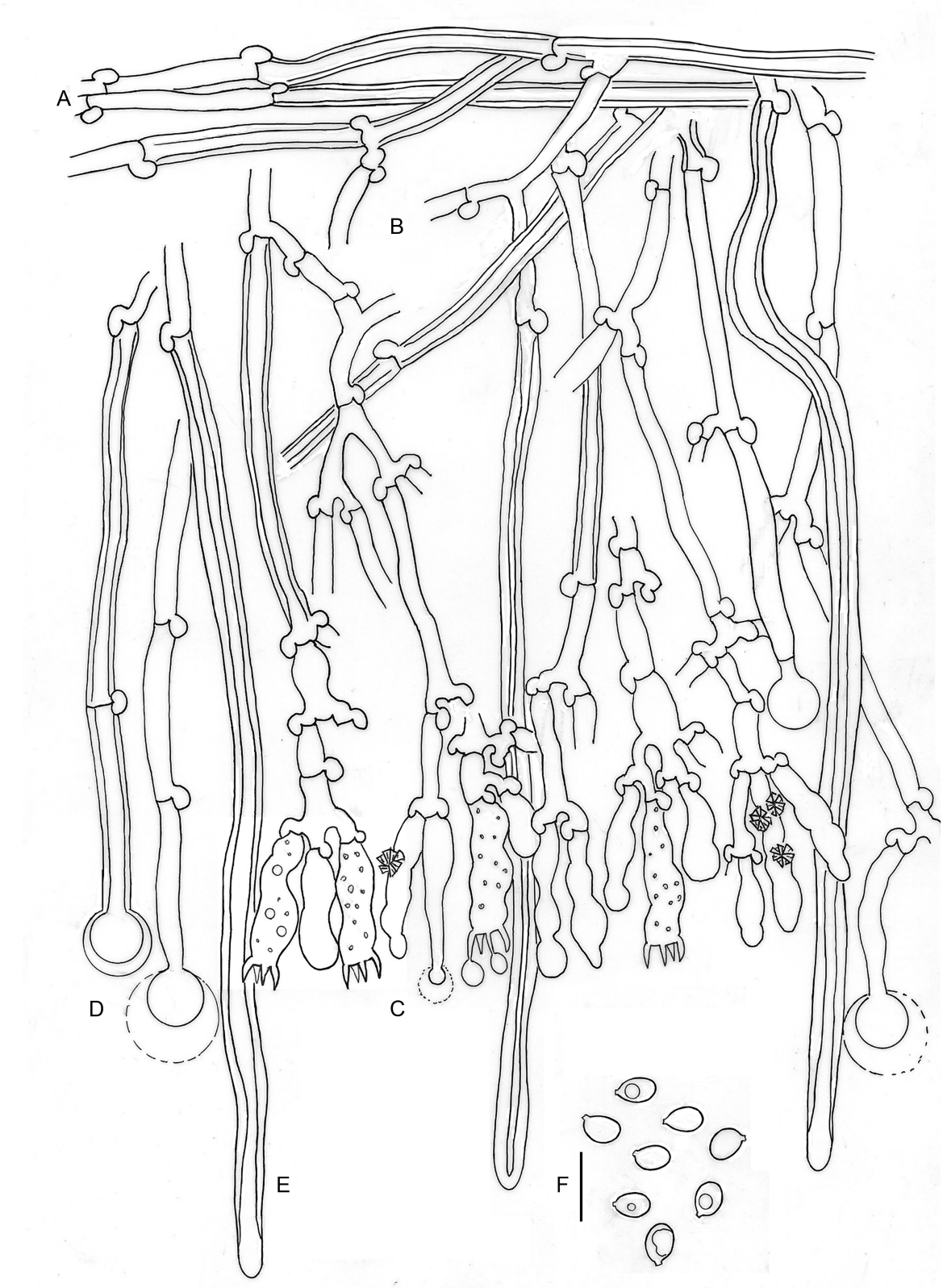

Fig 6. *Xylodon raduloides*, 002JFL, MA-Fungi 90709. A. Subicular hyphae. B. Subhymenial hyphae. C. Hymenium with cystidia and basidia. D. Capitate hyphae. E. Tubular hyphae or "skeletocystidia". F. Basidiospores. Bar=10 μm. Drawn by Margarita Dueñas.

(2.5–)3–3.5(–4.5) µm, hyaline, thin-walled, smooth, guttulate (Fig 6F). L=5.34, W=3.33, Q=1.60.

Ecology and habitat: On rotted wood of *Alnus*, *Carpinus*, *Castanea*, *Eucalyptus*, *Fagus*, *Quercus*, and *Tilia*. Present in areas with seasonal climate, warm and dry summers and cold winters.

Known distribution: Widespread in Europe, also known from Africa (Canary Islands and Cameroon).

Notes: *Xylodon raduloides* has been reported from northern Iran (Hallenberg, 1983) but we did not study any specimens from the Middle-East region.

The name *X. raduloides* unfortunately had to be introduced because when the combination *Xylodon radula* (Fr.) Tura et al. was made the basionym cited was that of Fries and not that of Persoon, which does not belong to this taxon but to *Basidioradulum radula* (Fr.) Nobles. Even though the name may have been inadvertently misapplied, the combination into *Xylodon* was nevertheless validly published and has to be maintained and applied in accordance with its type, unless a formal conservation proposal was made and eventually accepted. As the name *X. raduloides* has already been introduced to deal with the situation, in order to avoid further possible confusions we retain *X. raduloides* here.

Material examined: CAMEROON, Sakbayeme, 29 Apr. 1980, Rev. *Chas. Schwab*, (NY s.n., as *Schizopora subiculoides*). CANARY ISLANDS, Tenerife: Esperanza forest, on Eucalyptus, 17 Jan. 1990, *R Korf* (MA-Fungi 35643). FRANCE, Moselle, Monterhouse, Canton de Bitche, Forêt Domainale de Monterhouse (parcéle M-43), 280 msl, on dead wood, 25 Oct. 2009, *I. Melo, I. Salcedo & M.T. Telleria* 12028IS (MA-Fungi 79442); Moselle, Parc Naturel des Vosges du Nord, Pays de Bitche, Forêt Domaniale de Haut III, Rothenbruch Reserve, 49º01'00"N 7º35'50"E, 250 msl, on *Fagus sylvatica*, 29 Oct. 2009, *I. Melo, I. Salcedo & M.T. Telleria* 18336Tell. (MA-Fungi 79314); Pyrénées-Orientales, Languedoc-Rosillon, Massif des Albères, Lavall, 42º30'27"N 3º00'18"E, 225 msl, on *Quercus suber*, 5 Nov. 2008, *M. Dueñas, I. Melo, I. Salcedo & M.T. Telleria*, 11851IS (MA-Fungi 78658); Seine-et-Marne, Fontainebleau, Réserve Integrale, Gorge aux Loups (parcéle 527), 90 msl, on *Fagus sylvatica*, 30 Oct. 2006, *M. Dueñas, I. Melo, I. Salcedo & M.T. Telleria*, 11074MD (MA-Fungi 70457). SPAIN, Asturias, Reserva Biológica de Muniellos, on *Quercus robur*, 15 June 1983, *N. Brito, F.D. Calonge, M. Dueñas, V. Pou & M.T. Telleria* 755MD (MA-Fungi 12864); Ávila, Gavilanes, 40º13'18"N 4º50'15"W, on *Quercus ilex*, Nov. 2015, *J. Fernández-López* 002JFL (MA-Fungi 90709); Cantabria, Potes, Monte Tolibe, 450 msl, on *Quercus suber*, 1 Apr. 1985, *P. Coello, M. Dueñas, K.*

Escalante & M.T. Telleria 6996Tell. (MA-Fungi 12877); Ciudad Real, Fuencaliente, Robledo de las Ollas, 770 msl, on *Quercus suber*, 12 Apr. 2007, *F. Prieto & A. González* GP2291 (MA-Fungi 75310); ibid., Valle de la Cerceda, 880 msl, on *Quercus pyrenaica*, 16 Dec. 2004, *F. Prieto, A. González & al.* GP2162 (MA-Fungi 75244); ibid., 18 Nov. 2005, *F. Prieto, A. González & al.* GP2241 (MA-Fungi 75130); Huelva, El Barraco, Coto de Doñana, on *Quercus suber*, 24 Nov. 1977, *F.D. Calonge* (MA-Fungi 608); Palencia, Cervera de Pisuerga, on *Quercus pyrenaica*, 20 Nov. 1984, *N. Brito, M. Dueñas & M.T. Telleria* 2266MD (MA-Fungi 12778); Toledo, between Fresnedilla and El Real de San Vicente, on *Castanea sativa*, 29 May 1988, *M. Dueñas* 4719MD (MA-Fungi 22499); idem, 4736MD (MA-Fungi 22513); ibid., Velada, los Baldíos, río Guadyerbas, 395 msl, on *Quercus faginea*, 28 Mar. 2006, *F. Prieto, A. González & F.D. Calonge* GP2253 (MA-Fungi 75272).

Xylodon laurentianus J. Fernández-López, Telleria, M. Dueñas, & M.P. Martín, **sp. nov.** MycoBank MB288019 (Figs 5B-C and 7)

Etymology: Named after Laurentia, the ancient geological core (craton) of the North American continent, where the species is distributed.

Diagnosis: Morphologically this species is similar to *Xylodon raduloides*, but can be distinguished by the narrowly ellipsoid to subcylindrical basidiospores, (4.5–)5–6 × 2.5–3.5 µm (Q=1.70), instead of the ellipsoid ones, (4.5–)5–5.5(–7) × (2.5–)3–3.5(–4.5) µm (Q=1.60) in *X. raduloides*.

Type: USA, Washington DC, Ruch Drive, Rock Creek Park, on Quercus log, 18 June 1968, *H.H. Burdsall Jr.* HHB-719 (CFMR – holotype; Forest Products Laboratory (USDA) – ex-type culture: ITS, LSU and tef-1 sequences GenBank KY962845, KY962865, and KY967076).

Description: Basidioma resupinate, effuse, adnate; hymenophore poroid to labyrinthiform, 1–4 pores/mm, dissepiments lacerate to dentate in old specimens, on vertical substrata irregularly irpicoid with flattened teeth, orange-yellow (70. l. OY – 71. m. OY); margin not clearly differentiated. Hyphal system monomitic; generative hyphae hyaline, thin to thick-walled, sparsely branched, with clamps, 3–5 µm wide; subicular hyphae not seen; subhymenial hyphae loosely interwoven, perpendicular to substratum. Cystidia or rather cystidial elements present: (1) capitate and subulate cystidia, sometimes encrusted, arise from the hymenium, thin-walled, with basal clamp, 14–32 × 3.5–5 µm; (2)

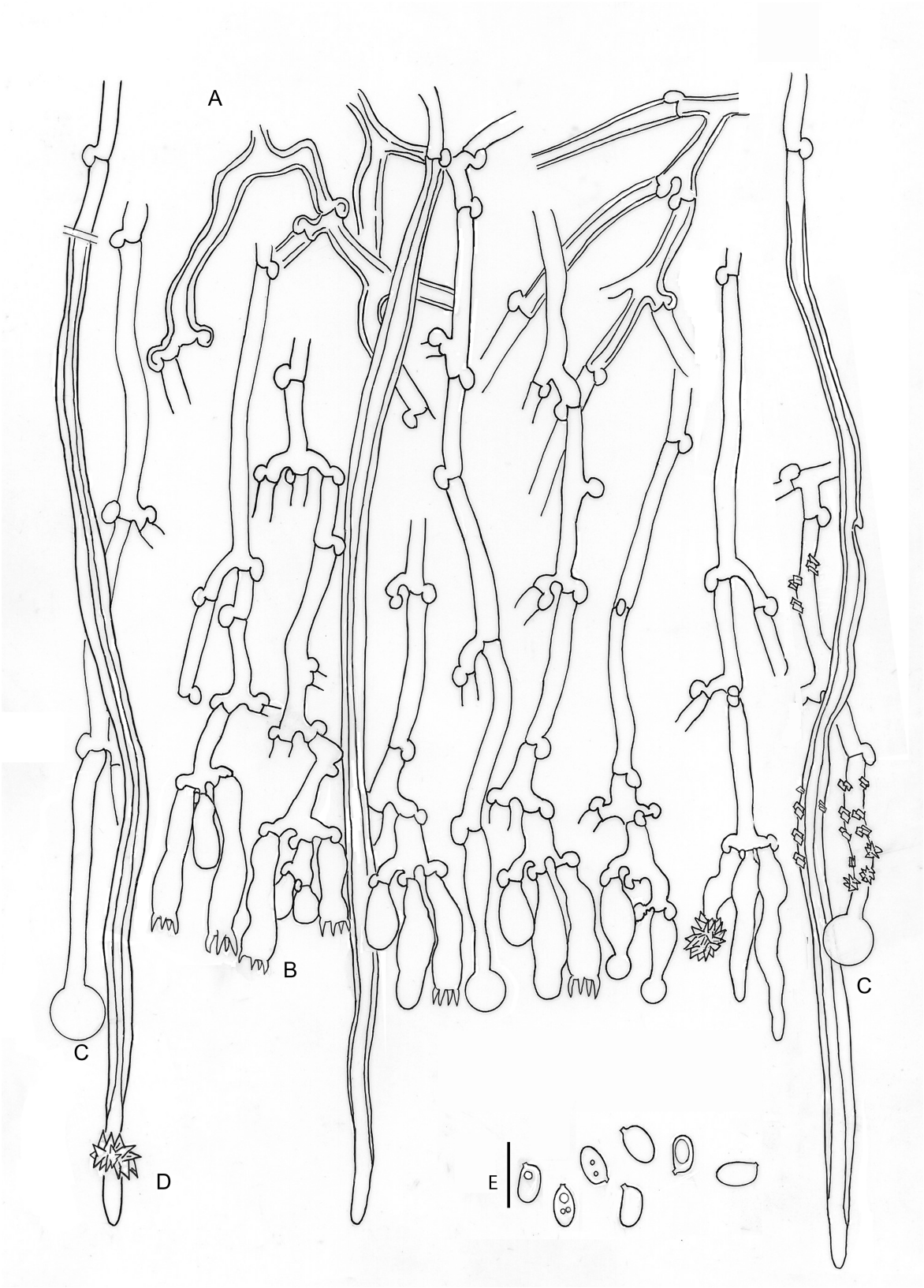

Fig 7. *Xylodon laurentianus*, HHB-719, CFMR, holotype. A. Subhymenial hyphae. B. Hymenium with cystidia and basidia. C. Capitate hyphae. D. Tubular hyphae or "skeletocystidia". E. Basidiospores. Bar=10 μm. Drawn by Margarita Dueñas.

capitate hyphae sometimes encrusted, thin-walled, with basal clamp, 25–46 × 3–4 µm, apex to 8 µm diam; and (3) tubular hyphae or "skeletocystidia" sometimes encrusted, with very thick walls narrowing to the apex, with basal clamp, 170–200 × 3.5–5.5 µm. Basidia cylindrical to suburniform, (13–)18–26 × 4.5–5.5 µm, four sterigmata, with basal clamp. Basidiospores narrowly ellipsoidal to subcylindrical, (4.5–)5–6 × 2.5–3.5 µm, hyaline, thin-walled, smooth, guttulate. L=5.22, W=3.08, Q=1.70.

Ecology and habitat: On dead wood of *Quercus*. Present in areas with a seasonal climate, warm and dry summers and cold winters.

Known distribution: Reported from Central and Eastern USA (Minnesota and Washington DC).

Additional material examined: USA, Minnesota, St Louis County, Independence, on dead wood, 28 Oct. 2009, *D.L. Lindner* DLL2009-049 (CFMR).

Xylodon patagonicus J. Fernández-López, Telleria, M. Dueñas, & M.P. Martín, **sp. nov.** MycoBank MB288018 (Figs 5D-F and 8)

Etymology: Named after Patagonia, the region where the holotype was collected.

Diagnosis: Morphologically similar to *Xylodon raduloides*, but differs in having smaller basidia, 13–18 × 3–4.5 µm, and shorter basidiospores, (3.5–)4–5.5(–6) × (2–)2.5–3.5(–4.5) µm with Q=1.46.

Type: Chile: Los Lagos (X Región): Palena, Comuna Hualaihué, Comau Fjord, Huinay, "Cementerio de Alerces", 42º21'57.9"S 72º24'56.9"W, 30 msl, on *Amomyrtus luma*, 29 Apr. 2012, *M. Dueñas, M.P. Martín & M.T. Telleria* 19684Tell (MA-Fungi 90707 –holotype; ITS, LSU and rpb2 sequences GenBank KY962837, KY962855, and KY967061).

Description: Basidioma resupinate, effuse, adnate, orbicular to confluent; hymenophore poroid to labyrinthiform, 1–5 pores/mm, dissepiments lacerate to dentate in old specimens, on vertical substrata irregularly irpicoid with flattened teeth, yellow-white to orange-yellow (92. y White – 71. m. OY); margin not clearly differentiated. Hyphal system monomitic; generative hyphae hyaline, thin to thick-walled, branched, with clamps, 2.5–4 µm wide; subiculum not clearly differentiated; subhymenial hyphae loosely interwoven, perpendicular to substratum. Cystidia or rather cystidial elements present: (1) capitate and subulate cystidia, sometimes encrusted, arise from the hymenium, thin-walled, with basal clamp, 17.5–25 × 3–4 µm; (2) capitate hyphae, thin-walled, basal clamp, 22–56 × 2.5–4 µm, apex up to 8 µm diam; and (3) tubular hyphae or "skeletocystidia" very thick-

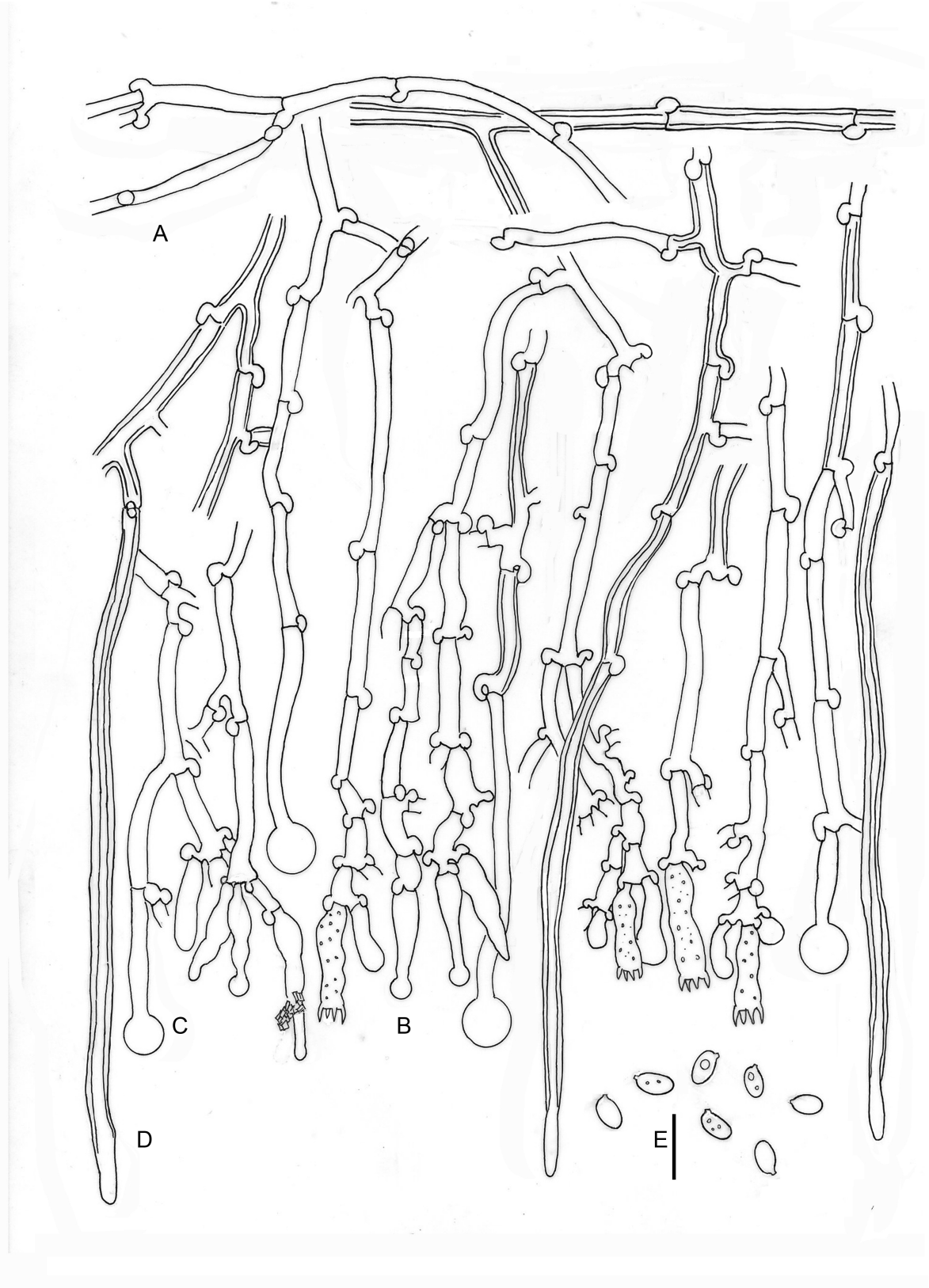

Fig 8. *Xylodon patagonicus*, 19684Tell., MA-Fungi 90707, holotype. A. Subhymenial hyphae. B. Hymenium with cystidia and basidia. C. Capitate hyphae. D. Tubular hyphae or "skeletocystidia". E. Basidiospores. Bar=10 μm. Drawn by Margarita Dueñas.

walled narrowing to the apex, basal clamp, 80–115 × 3–4 µm. Basidia cylindrical to suburniform, 13–18 × 3–4.5 µm, four sterigmata, with basal clamp. Basidiospores ellipsoidal, (3.5)4–5.5(–6) × (2–)2.5–3.5(–4.5) µm, hyaline, thin-walled, smooth, guttulate. L=4.56, W=3.11, Q=1.46.

Ecology and habitat: On dead wood of *Nothofagus nitida* and *N. dombeyi* (Nothofagaceae), and *Amomyrtus luma* (Myrtaceae). Present in areas with a mild climate, with low annual variations in temperature, and high humidity during dry season.

Known distribution: Reported from the Patagonian region (southern Chile and southern Argentina).

Notes: Poria platensis was described from Argentina by Spegazzini (1902) and later synonymized by Lowe (1963) with *Schizopora paradoxa*. Hallenberg (1983) segregated *Schizopora radula* (i.e. *Xylodon raduloides*) from *S. paradoxa* and therefore *P. platensis* could be related to the *X. raduloides* complex, and more specifically with *X. patagonicus*, but the substrate was given as on *Pinus* beams rather than on a hardwood so is unlikely to be conspecific. No specimens of this taxon were available to study and investigate this further.

Additional material examined: CHILE: Los Lagos (X Región), Palena, Comau Fjord, Comuna Hualaihué, Huinay, path to Cerro del Tambor, 42º22'53.2"S 72º24'44.0"W, 125 msl, on fallen logs, 26 Apr. 2012, *M. Dueñas, M.P. Martín & M.T. Telleria* 3341MPM (MA-Fungi 90704); idem, 3340MPM (MA-Fungi 90708); ibid., on *Nothofagus nitida* wood, 26 Apr. 2012, *M. Dueñas, M.P. Martín & M.T. Telleria* 14007MD (MA-Fungi 90705); ibid., path to Cerro del Tambor behind hydroelectric power station, 42º22'54.2"S 72º24'53.5"W, 202 msl, on fallen logs, 8 May 2013, *M. Dueñas, M.P. Martín & M.T. Telleria* 3567MPM (MA-Fungi 90703); ibid., "Derrumbe Antiguo", 42º22'17.0"S 72º24'12.2"W, 120 msl, on *Nothofagus dombeyi*, 1 May 2012, *M. Dueñas, M.P. Martín & M.T. Telleria* 14180MD (MA-Fungi 90702); ibid., Lloncochaigua river bank, near to the bridge, 42º22'09.0"S 72º24'42.7"W), 19 msl, on dead wood, 30 Apr. 2012, *M. Dueñas, M.P. Martín & M.T. Telleria* 19705Tell (MA-Fungi 90706).

Xylodon novozelandicus J. Fernández-López, Telleria, M. Dueñas, & M.P. Martín, **sp. nov.** MycoBank MB828020 (Figs 5G-H and 9)

Etymology: Named after New Zealand, where the holotype was collected.

Diagnosis: This species is morphologically similar to *Xylodon patagonicus*, but differs in having subcylindrical cystidia arising from the hymenium.

Type: NEW ZEALAND, Wellington, Kaitoki, Swingbridge Track, on dead wood in "Podocarp/Broadleaf forest", 22 Feb. 1998, *I.G. Steer & B.C. Paulus, B.C. Paulus* 98/20 (PDD 70718 – holotype; ICMP13838 – ex-type culture; ITS, LSU and tef-1 sequences GenBank AF145578, KY962851, and KY967069).

Description: Basidioma resupinate, effuse, adnate; hymenophore poroid to labyrinthiform, 1–4 pores/mm, dissepiments lacerate to dentate in old specimens, orange-yellow (70. l. OY); margin not clearly differentiated. Hyphal system monomitic; generative hyphae hyaline, thin to thick-walled, encrusted, branched, with clamps, 2.5–4.5 µm wide; subicular hyphae not seen; subhymenial hyphae loosely interwoven, perpendicular to the substratum. Cystidia or rather cystidial elements present: (1) capitate, subcylindrical and subulate cystidia, sometimes encrusted, arise from the hymenium, thin-walled, basal clamped, 13–27 × 3.5–4.5 µm; (2) capitate hyphae, thin-walled, with basal clamp, 28–34 × 2–3.5 µm, apex up to 9 µm diam; and (3) tubular hyphae or "skeletocystidia" very thick-walled narrowing to the apex, with basal clamp, 110–150 × 3.5–4.5 µm. Basidia cylindrical to suburniform, (9–)10–15 × 3–4.5 µm, four sterigmata, with basal clamp. Basidiospores ellipsoidal, 4–6 × (2.5–)3–4(–4.5) µm, hyaline, thin-walled, smooth, guttulate. L = 4.71, W = 3.21, Q = 1.47.

Ecology and habitat: Growing on dead wood identified as *Carpinus betulus* and *Fuscospora cliffortioides*. Present in areas with a mild climate, low annual variations in temperature, and high humidity during the dry season.

Known distribution: Reported from Australasia (Australia and New Zealand). Also found in France and Canada (British Columbia).

Notes: Timber trade and transport could easily explain this distribution pattern for both French and Canadian samples (Table 1, Fig. 3). No morphological study was carried out on the Canadian sample, since the ITS sequence was obtained from a culture. However, a morphological study of the French specimen revealed differences in spore morphology from New Zealand material, being similar to those from the European clade, so this trait could be linked to environmental conditions.

Additional material examined: FRANCE, Côtes-d'Armor, Commune de Plévenon, Saint Cast-le Guildo, La Fresnaye, 48º38'33.7"N, 2º16'31.7"W, 15 msl, on *Carpinus betulus* wood, 24 Oct. 2010, *M. Dueñas, I. Melo, I. Salcedo & M.T. Telleria* 12836IS (MA-Fungi 74919). NEW ZEALAND, Buller, South of Punakaiki Field Centre, "Lifestyle Blocks", on fallen branches, 16 May 1998, *I.G. Steer & B.C. Paulus, B.C. Paulus* 98/81 (PDD 70716; ICMP 13841 – culture); Mid-Canterbury, on Fuscospora cliffortioides wood, 11

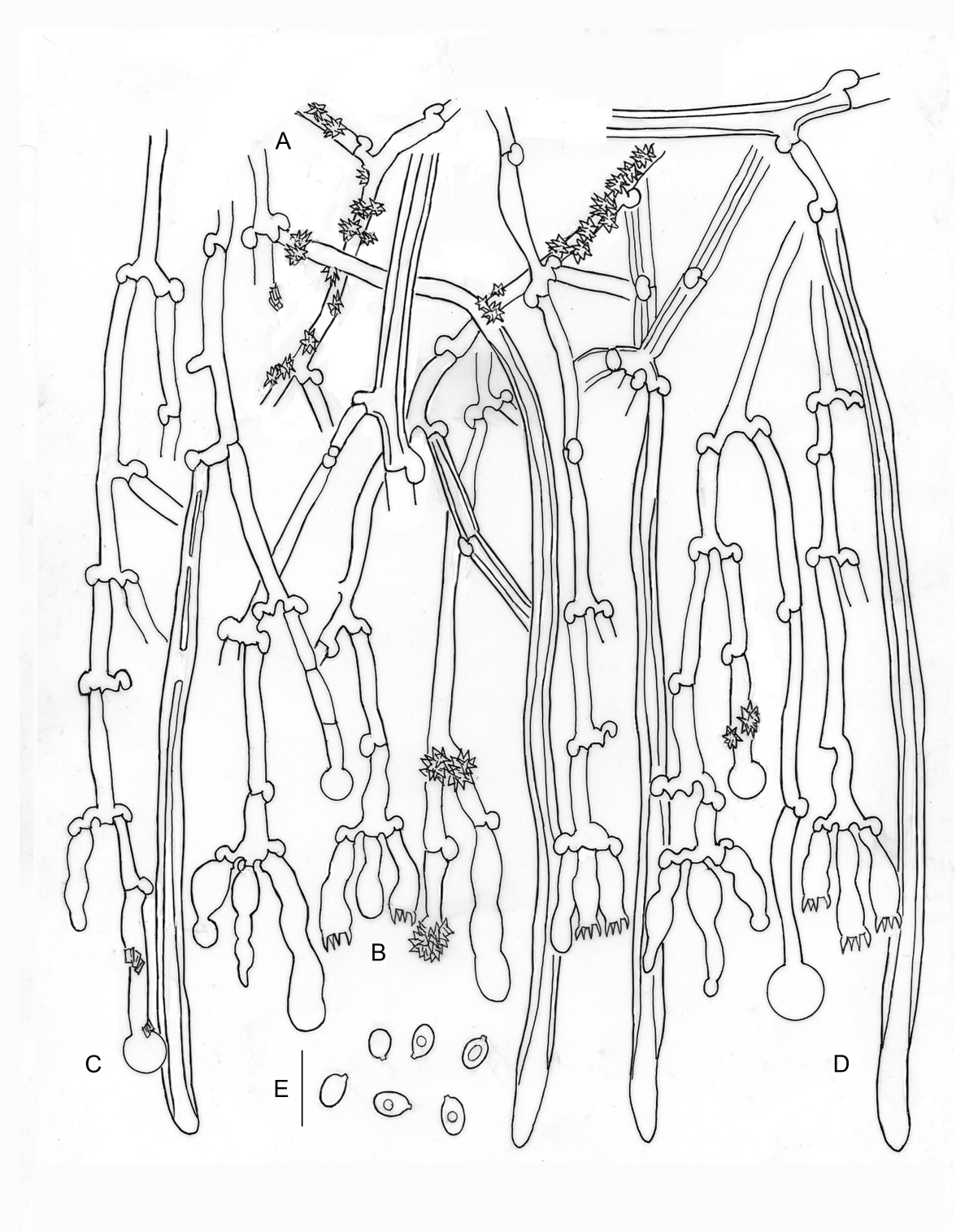

Fig 9. *Xylodon novozelandicus*, Paulus 98/20, PDD 70718, holotype. A. Subhymenial hyphae. B. Hymenium with cystidia and basidia. C. Capitate hyphae. D. Tubular hyphae or "skeletocystidia". E. Basidiospores. Bar=10 μm. Drawn by Margarita Dueñas.

May 2006, *A. Roberts & B.C. Paulus* (PDD 91616); ibid., Christchurch, Riccarton Bush, fallen branch, 17 May 1998, *I.G. Steer & B.C. Paulus, B.C. Paulus* 98/104 (PDD 70720; ICMP 13840 – culture).

DISCUSSION

Morphological species recognition has limits in a group like the *Xylodon raduloides* species complex; alone, it would not likely identify the species and geographic diversity revealed during this study. Our ITS and LSU analyses revealed four species within the *X. raduloides* complex (Fig 2). These species were confirmed in a multi-locus coalescent framework, since the Bayes factors approach further established the ITS species proposal (hypothesis-C) as the most probable given the data. The ability of ITS sequence data to detect hidden diversity in fungi has been questioned leading to the suggestion that a multi-locus approach should be used (Balasundaram et al. 2015). Our results suggest that the ITS region performs well for the *X. raduloides* species complex, but this is likely context dependent so general inferences about its utility in species delimitation should be cautious as they may strongly depend on the group being studied (Balasundaram et al. 2015, Wilson et al. 2017a).

Although the ITS region is a powerful tool for discriminating among fungal species, as it is a non-transcribed, non-coding region makes it prone to accumulate homoplasies (Nagy et al. 2012). This accumulation of random homoplasies means the ITS region alone is not generally useful to study inter-species relationships. Thus, the multi-locus species coalescent approach allowed for comparison of relationships among species since internal nodes showed high support for geographic relationships (Fig 3). Salgado-Salazar et al. (2013) used this method to separate 16 distinct highly supported lineages in the morphologically circumscribed *Thelonectria discophora* which were linked to different geographic areas and ecological settings.

The multi-locus phylogenetic approach, which incorporated rpb2, and tef-1α along with ITS and LSU sequence data, revealed that Holarctic taxa (*X. raduloides* and *X. laurentianus*) were more genetically related, and that *X. patagonicus* and *X. novozelandicus* shared a recent common ancestor (Fig 3). While it has been demonstrated that fungal distributions can be human-mediated (Fuller et al. 2013), only two specific instances of possible human translocation are supported by our results: specimens of *X. novozelandicus* were reported from France and Canada. As a result, the extant

biogeographic distribution of the *Xylodon* species studied is likely due to natural processes.

Our results correlate with geography and suggest allopatric differentiation within the *Xylodon* species in this study, confirming the proposal of Paulus et al. (2000). This geographic phylogenetic structure has been observed in other basidiomycetes, like the *Schizophyllum commune* complex (James et al. 2001), the genus Lentinula (Hibbett 2001) and the lethal amanitas (*Amanita* sect. Phalloideae; Cai et al. 2014). This reveals the importance of geographic separation in genetic isolation and gene flow in fungi (Taylor et al. 2006). In other cases, such as in *Laccaria* (ectomycorrhizal fungi), in addition to geographic barriers, a study of host associations is necessary to obtain a proper understanding of the factors that explain species distributions (Wilson et al. 2017b). These results show that multiple factors can affect the biogeographical patterns in fungi.

Biogeographical patterns shown by the Southern Hemisphere species agree with the general pattern observed for most Gondwanan groups of plants (Sanmartín & Ronquist 2004). A deep vicariance event could be inferred between *X. patagonicus* and *X. novozelandicus*, which could be due to the geological breakup of the supercontinent Gondwana approximately 80 MYA (Scotese et al. 1988). Molecular differences were not found between Australian and New Zealand specimens of *X. novozelandicus*, suggesting the absence of genetic isolation. Dispersal events between Australia and New Zealand may well explain how a single species in these two areas is maintained, while remaining genetically isolated from *X. patagonicus*. This dispersal ability has been commonly observed in Southern Hemisphere plants (Seberg 1991; Linder & Crisp 1995; Knapp et al. 2005) and fungi (Moncalvo & Buchanan 2008; Peterson et al. 2010; Wilson et al. 2017b).

The close relationship between woody plants and corticioid fungi suggests a shared historical biogeography. In this context, little is known about host specificity for the *X. raduloides* complex. A variety of hosts (*Alnus*, *Carpinus*, *Quercus*, *Tilia*, etc.) have been reported in the European region (Langer 1994; Ryvarden & Melo 2014), while for the New Zealand region it has been reported on decayed southern beech: *Fuscospora cliffortioides*, *F. fusca*, *Lophozonia menziesii* (Clinton et al. 2009). In general, current knowledge points toward a broad range of hosts for the *X. raduloides* complex, which could account for the worldwide distribution.

The geographic and phylogenetic patterns confirm that for the *X. raduloides* complex, as in other basidiomycetes (Hibbett 2001), the hypothesis “everything is everywhere” is not applicable. The traditional dependence on morphological species

recognition criteria has led to an underestimate of species diversity and did not reveal the actual distribution patterns for the *Xylodon* species in our study.

Morphological analysis of diversity in the *X. raduloides* complex confirms that basidiospore morphology may be only partially effective as an inter-specific diagnostic character in these fungi. Spore shape (length to width ratio, Q) was able to distinguish between Northern and Southern Hemisphere groups (Fig 4). Northern Hemisphere specimens have longer spores, while spores of Southern Hemisphere species have a more spherical shape. No statistical differences were found among intra-hemisphere specimens (Fig 4). This observation could be due to the close phylogenetic relationship within Northern and Southern Hemisphere species, supported by our molecular results (Figs 2–3). While little attention has been paid to spore morphology (Parmasto & Parmasto 1992), their importance as dispersion propagules, in sexual reproduction and gene flow in fungi (Kauserud et al. 2008) makes them an informative diagnostic character even when morphological stasis is observed for other traits. However, spore morphology may be insufficient to discriminate recent speciation in the inter-Hemisphere *X. raduloides* complex.

Environmental niche analyses performed for the species complex showed non-equivalence between Northern and Southern Hemisphere species niches (Fig. 3). Bioclimatic associations also were in concordance with molecular data and separate the complex by Hemisphere. Environmental traits that defined these two groups could be summarized in an isothermal-seasonal gradient. Northern Hemisphere species are acclimated to a more seasonal environment, with warmer and drier summers and colder winters; while Southern Hemisphere species fructified in mild climates, characterized by low annual thermal variations and more humidity during the dry season. These results indicate that phylogenetically related species occupy similar environmental niches.

The correlation between spore morphology and environmental features in the *X. raduloides* complex is interesting. There is a demonstrated relationship between spore morphology and environmental conditions in many other fungi (Kauserud et al. 2008). The metabolic costs of spore production make it subject to evolutionary fitness (Stearns 1992). Larger spores are correlated with more seasonal areas; this association could be explained by the necessity of storing more nutrients for the transition from dry season to sporophore production (Kauserud et al. 2011). This hypothesis is in agreement with our results for the *X. raduloides* complex, since the Northern Hemisphere group –with a more seasonal climate– showed spores of greater volume by virtue of being longer than the spores from

the Southern Hemisphere species (Fig 4). Whether or not the concordance between environmental characteristics and spore morphology is indicative of a cause and effect relationship remains to be evaluated in this complex, since the correlation between environmental and morphological traits could also be explained by the shared evolutionary and geographic history between sister species. Further phylogenetic comparative analysis, e.g. assessing the phylogenetic signal for spore morphology or environmental preferences (Felsenstein 1985; Grafen 1989; Revell et al. 2008) should be conducted to specifically test these hypotheses.

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Chapter 3

New tools for old problems: the case of *Xylodon australis*

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ABSTRACT

The use of different sources of evidence has been recommended in order to conduct species delimitation analyses as well as to solve nomenclatural and taxonomic issues. However, the combination of those sources is not always straightforward. Here, we use a maximum likelihood framework to combine morphological and molecular traits to study the *Xylodon australis* (Hymenochaetales, Basidiomycota) species complex using the *locate.yeti* function from the *phytools* R package. *Xylodon australis* has been considered a single species distributed across Australia, New Zealand and Patagonia. Multi-locus phylogenetic analyses were conducted to unmask the actual diversity under *X. australis* as well as the kinship relationships with respect to their relatives. To assess the taxonomic position of each clade, *locate.yeti* function was used to locate in a molecular phylogeny the *X. australis* type material for which no molecular data was available using morphological continuous traits. Two different species were distinguish under the *X. australis* name with a strong biogeographic pattern, one from Australia–New Zealand and other from Patagonia. In addition, a close relationship with *Xylodon lenis*, a species from the South East of Asia, was confirmed for the Patagonian clade. We discuss the implications of our results for the biogeographical history of this genus as well as we evaluate the potential of this method to be used with historical collections for which destructive sampling or to obtain molecular data is not available.

INTRODUCTION

Only six years before the famous wreck of the HMS Erebus and HMS Terror during Franklin's lost Arctic expedition, Sir James Clark Ross commanded the same two vessels during his Antarctic mission with the purpose of investigating terrestrial magnetism between 1839 and 1843. Onboard the HMS Erebus was the British botanist Sir Joseph Dalton Hooker, enrolled as assistant ship's surgeon and to collect natural history specimens. During four years they explored many austral areas from New Zealand and Tasmania to the Antarctic and Tierra de Fuego, including numerous islands, such as Crozet, Kerguelen or Falkland, collecting samples from species never described before. Most of the plant and fungal specimens from this expedition were deposited in the Kew Herbarium and many new fungus species were described by Miles Joseph Berkeley in Hooker's work "*The Botany of the Antarctic Voyage*" (1860).

Among these new species described by Berkeley, *Grandinia australis* Berk. [≡*Xylodon australis* (Berk.) Hjortstam & Ryvarden] was collected from Tasmania Island. This species is a white rot corticioid fungus characterized by its chestnut-orange hymenial surface that turns violet upon the application of KOH. This longtime neglected species was only known from Australia (Hjortstam 1995) but, in recent decades, Greslebin et al. (2000) reported this species from Argentina and New Zealand, extending its known distribution. Though they found morphological differences in basidiospores among samples from different areas, they maintained the specimens studied from New Zealand, Tasmania and Argentina as a single species with an Austral distribution.

In recent decades, there has been a shift in the criteria to identify fungal species. The phylogenetic species recognition (PSR), based on the analyses of DNA sequences, has shown a closer match to an evolutionary species concept than other methods such as the traditional morphological species recognition (MSR) or the biological species recognition, BSR (Taylor et al. 2000). As a result, there has been an increase of new species described based on DNA data in recent years. This shift toward the phylogenetic species recognition has been possible due to the development of molecular tools to obtain DNA data. Initiatives such as Assembling the Fungal Tree of Life, AFToL (McLaughlin et al. 2009) or those carried out by the Fungal Barcode Consortium (Schoch et al. 2012) have led to the identification of the best DNA regions for phylogenetic reconstructions or for new fungal species identification.

The revision of old species names and the study of type materials are necessary in order to ensure the correct taxonomic classification of biodiversity. However, when DNA sequences are used as the key evidence in the study of fungi, the lack of molecular data from type collections can be a problem. Type specimens are often old, dry material deposited in herbaria where DNA can be poorly preserved, or specimens can be treated with fixatives that have damaged DNA. Although it has been possible to extract and amplify the nuclear ribosomal Internal Transcribed Spacer region (ITS) fungal barcode (Schoch et al. 2012), from very old specimens, like the case of a specimen of *Agaricus cossus* collected in 1794 (Larsson & Jacobsson 2004), or another specimen of *Hyphodermella rosae* from 1926 (Telleria et al. 2010), this is an exception. The success in DNA amplification usually decreases with the specimen age, making it difficult to obtain enough high quality DNA to be used in phylogenetic studies (Drábková 2014). In addition, some herbaria have special policies about type specimens, and destructive sampling to obtain DNA data is not always allowed due to the historical value of those collections (Dayarathne et al. 2016). This is the case of *Xylodon australis* type material, collected during the Ross Antarctic Expedition, which is available in the herbarium of the Royal Botanic Gardens Kew (K) for morphological study, but not for destructive sampling.

Since DNA data have emerged as a vital source of information to identify fungal species and to study their diversity, when molecular data of type materials is missing the reliability of their classification and/or nomenclature may be compromised. However, in many cases, additional morphological data or geographic origin can help to assign type specimens to a specific clade obtained from molecular phylogenetic analyses.

In recent years, several methodological approaches have been deeloped to include fossils or recently extinct taxa in molecular phylogenies using morphological characters (Revell et al. 2014; Parins-Fukuchi 2018). These tools are based on modifications of Felsenstein's approach (Felsenstein 1973, 1981) to estimate phylogeny from continuous characters. Starting with an ultrametric molecular phylogeny for N–1 species and a continuous character dataset, these methodologies are able to infer the position of a new taxon not present in the molecular phylogenetic tree, from the measurements of particular phenotypic characters (Revell et al. 2014). The same scheme can be applied to locate a type specimen in a molecular phylogenetic tree in order to solve taxonomic issues when DNA sequences are used in the study of fungi or other organisms.

Here, we address the case of the *Xylodon australis* complex using the methodologies described above. In order to assess the actual diversity of *X. australis*, two-

loci phylogenetic analyses were conducted. No molecular data were obtainable from the nomenclatural type specimen, since destructive sampling was not allowed, due to its historical value. Thus, morphological studies were carried out to place the type material into the molecular phylogenetic tree, using a maximum likelihood framework through the *locate.yeti* function from the *phytools* R package to solve the possible taxonomical issues (Revell 2012).

MATERIALS AND METHODS

Taxon sampling and morphological studies

A total of 37 specimens of *Xylodon australis* from three different herbaria: Australian National Herbarium (CANB), New Zealand Fungal & Plant Disease Collection (PDD), Real Jardín Botánico from Madrid (MA-Fungi); and Alina Greslebin and Mario Rajchenberg private collections, were analyzed in this study (Table 1). In addition, the type material of *X. australis* (under *Grandinia australis* Berk.) from Royal Botanic Gardens Kew (K) was also studied morphologically. Basidioma colors were recorded according to Kelly & Judd (1976). Color changes were examined with 3% aqueous KOH. Microscopic measurements were made from sections mounted in aqueous solutions of 3% KOH and 1% aqueous solution of ammoniacal Congo red or 1% aqueous floxine. Sections were examined at magnifications up to 1250× using an Olympus BX51 microscope. Six basidia were measured from each sample. The width (W) and length (L) of 10 spores were also measured and length/width ratios (Q) were calculated. Average values of each character were calculated for each specimen. Additional morphological measurements were performed to provide a general description of each species. Drawings were made with the aid of a drawing tube.

DNA extraction, amplification and sequencing

Genomic DNA isolation was performed using DNeasy™ Plant Mini Kit (Qiagen, Valencia, California, USA) following the manufacturer's instructions, except in three steps: the incubation with the RNAase was done overnight at 65 °C, a second drying at 20.000 × g was done for 2 minutes after cleaning with AW buffer, and elution buffer was preheated to 60 °C. When this extraction was not successful, FTA® Indicating Micro Cards (Cat Nº

Table 1. Specimens included in the morphological analysis. Accesion numbers in bold are from newly generated sequences. L=Length, W=Width, Q=Length/Width ratios, * (-)=Not observed; ** (-)=Not obtained.

Species /specimens	Country	Basidia morphology*		Basidiospore morphology*			GenBank Acc. N°**	
		L	W	L	W	Q	ITS	LSU
Xylodon australis (Berk.) Hjortstam & Ryvarden								
K 56442 (Holotype)	Australia	29.8	5.1	7.2	5.1	1.41	-	-
CANB569566	Australia	28.58	4.75	7.3	4.6	1.59	**MT158734**	-
CANB569567	Australia	32	4.83	6.8	4.4	1.55	**MT158703**	**MT158739**
CANB569568	Australia	28	4.3	6.3	4.2	1.50	-	-
CANB569570	Australia	26.5	4.75	6.7	4.3	1.56	**MT158735**	-
CANB569572	Australia	30	4.7	7.3	4.4	1.66	-	-
CANB751963	Australia	28.7	4.97	6.4	4.2	1.52	**MT158708**	**MT158744**
CANB752080	Australia	29.65	4.5	7	4	1.75	**MT158709**	**MT158745**
CANB752088	Australia	28.3	4.7	6.7	4.1	1.63	**MT158710**	**MT158746**
CANB869100	Australia	28.85	5	6.5	4	1.63	**MT158715**	**MT158751**
CANB869124	Australia	30.3	5	6.3	4.1	1.54	**MT158716**	**MT158752**
PDD 23689	New Zealand	28.5	4	7.1	4.6	1.54	-	-
PDD 23691	New Zealand	30.56	5	6.8	5.2	1.31	-	-
PDD 23692	New Zealand	29.75	4.85	6.85	5.2	1.32	-	-
PDD 23693	New Zealand	31	4.75	6.8	4.9	1.39	-	-
PDD 23694	New Zealand	29.8	4.5	6.6	4.6	1.43	-	-
PDD 23696	New Zealand	30.45	4.67	6.1	4	1.53	-	-
PDD 23698	New Zealand	30.66	4.33	6.6	4.5	1.47	-	-
PDD 23699	New Zealand	26.33	4.66	6.7	4.4	1.52	-	-
PDD 23703	New Zealand	25.83	4	6.9	5	1.38	-	-
PDD 23704	New Zealand	29.5	4	6.4	4.7	1.36	-	-
PDD 23705	New Zealand	30.1	4.7	6.2	4.4	1.41	-	-
Xylodon lenis Hjortstam & Ryvarden								
Wu 890714 (Isotype)	Taiwan	18.5	3.75	4.75	3.25	1.46	KY081802	-
Xylodon magallanesii *sp. nov.*								
AG 730	Argentina	22.65	4	5.6	3	1.87	**MT158730**	**MT158766**
AG 1548	Argentina	23.75	4.62	6.1	2.9	2.10	**MT158731**	**MT158767**
AG 1872	Argentina	23.22	4.35	6	3	2.00	**MT158732**	**MT158768**
MA-Fungi 90397, 20008 Tell (Holotype)	Chile	24.5	4.75	5.6	2.87	-	**MT158729**	**MT158765**
MA-Fungi 90391, 14120 MD	Chile	21.4	4	6.4	3	2.13	**MT158720**	**MT158756**
MA-Fungi 90392, 14163 MD	Chile	23	4	5.8	3	1.93	-	-
MA-Fungi 90393, 14164 MD	Chile	22.5	4.5	6	3	2.00	**MT158721**	**MT158757**
MA-Fungi 91815, 14629MD	Chile	22.5	4.25	5.54	2.45	2.26	**MT158722**	**MT158758**
MA-Fungi 91816, 15630MD	Chile	27	4	5.1	2.65	1.92	**MT158723**	**MT158759**
MA-Fungi 91817, 15632MD	Chile	23.3	3.83	5.95	2.77	2.14	**MT158724**	**MT158760**
MA-Fungi 91818, 15634MD	Chile	-	-	5.17	2.50	2.06	**MT158725**	**MT158761**
MA-Fungi 91819, 15637MD	Chile	24	4.16	5.6	2.45	2.28	**MT158726**	**MT158762**
MA-Fungi 91820, 15638MD	Chile	-	-	-	-	-	**MT158727**	**MT158763**
MA-Fungi 91821, 20007Tell	Chile	23	4.5	5.72	2.90	1.97	**MT158728**	**MT158764**
PDD 69093, MR 11041	Argentina	21.85	4	6.3	3	2.10	**MT158733**	**MT158769**

WB120211, Whatman, Maidstone, England) were used following the protocol in Telleria et al. (2014). DNA amplifications, purifications and sequencing protocols are deposited in protocols.io (dx.doi.org/10.7504/protocols.io.wpdfdi6).

Polymerase chain reactions (PCR) were performed to amplify DNA from two loci using the following primer combinations: ITS5/ITS4 (White et al. 1990) were used to obtain DNA amplifications of the nuclear ribosomal internal transcribed spacer regions ITS1 and ITS2, including 5.8 S (ITS barcode, Schoch et al. 2012) and LROR/LR7r for LSU region (1-1583) (Rehner & Samuels 1994; Vilgalys & Hester 1990). When these pairs of primers failed, both regions were amplified in two parts: in the case of ITS, the region ITS1, including part of the 5.8S, with primers ITS5 and ITS2 (White et al. 1990), and part of 5.8S and the region ITS2 with primers ITS3 (White et al. 1990) and ITS4; in the case of LSU, one region between the pair of primers LROR and LR5 (White et al. 1990) and another region between the primers LR3R (Vilgalys & Hester, 1990) and LR7r were amplified. When neither direct nor amplification by parts gave good amplicons (above 20 ng/µl concentration), two semi-nested or nested PCR was used. For ITS, a first amplification was done with ITS1F (Gardes & Bruns, 1993) and ITS4B (Gardes & Bruns, 1993) primers, amplifying part of the 18S and 28S nuclear ribosomal genes, and a second amplification was done with the pair of primers ITS5/ITS4 (nested-PCR) or only one of the inner primer and one external primer (semi-nested PCR). For LSU the first amplification was done with LROR and LR7r primers, and the second amplifications were done with LROR/LR5, and LR3R/LR7r primers. Individual reactions to a final volume of 25 µl were carried out using IllustraTMPuReTaqTMReady-To-GoTM PCR Beads (GE Healthcare, Buckinghamshire, UK) with a 10-pmol µl-1 primer concentration following the thermal cycling conditions used in Martín and Winka (2000). Negative controls lacking fungal DNA were run for each experiment to check for contamination.

The PCR products were subsequently purified using two different methods. When the quality of the DNA was low, due to the presence of multiple bands, QIAquick Gel Extraction kit (QiaGen®) was used following manufacturer's instructions. When the quality of the DNA was high (a unique amplicon of above 20 ng/µl concentration), purifications were done using Exosap, IllustraTM ExoStar-1-Step (GE Healthcare, Buckinghamshire, UK) following the instructions of the manufacturers. Purified amplicons with a concentration of 20 ng/µl or more were sent to Macrogen (Korea) for sequencing with primers used in the amplification.

Phylogenetic analyses

Consensus sequences were obtained using Geneious version 9.0.2 http://www.geneious.com (Kearse et al. 2012). Subsequently, they were subjected to a BLAST search with megablast option and compared against the sequences in the National Center for Biotechnology Information (NCBI) nucleotide databases (Altschul et al. 1990) to check for contamination. Evaluation of EMBL/GenBank/DDBJ databases for ITS and LSU sequences of a large set of *Xylodon* species was performed to provide a phylogenetic framework to *X. australis* and to maximize the molecular information available for these taxa (Table S1). The maximum parsimony (MP), maximum likelihood (ML), and Bayesian Inference (BI) analyses of specimens in the S1 Table are also deposited in protocols.io under the doi mentioned above. The ML and Bayesian analyses were done with the general time reversible model (Rodríguez et al. 1990), including estimation of invariant sites and a discrete gamma distribution with six categories (GTR+G), as selected by PAUP*Version 4.0b10.

Individual datasets of ITS, LSU and a combined alignment of ITS + LSU regions were used to compare specimens of *Xylodon australis* from South America and Australia with other *Xylodon* species.

Statistical tests for morphological traits

Basidia and spore morphology were analyzed in order to assess the morphological difference found in previous studies for *X. australis* specimens from different locations (Greslebin et al. 2000). One-way ANOVA tests were performed between *X. australis* lineages determined by molecular phylogenetic analyses.

Inferring the position of the nomeclatural type of Xylodon australis in the molecular phylogenetic tree

In order to determinate the position of the type specimen of *Xylodon australis* in the molecular phylogenetic tree using phenotypic traits, we used the methodology proposed by Revell et al. (2014). First, ultrametric phylogenetic trees of *X. australis* s.l. and closely-related species were estimated using BI implemented in BEAST v2.4.3 (Drummond & Rambaut 2007; Bouckaert et al. 2014) for each alignment. Site model partition and

GTR+G substitution model was selected individually for both, ITS and LSU regions in all datasets using BEAUti v2.4.3 interface (Bouckaert et al. 2014). Birth-Dead model was used as tree prior. Three independent MCMC runs were specified for 50 million generations, sampling every 5000th generation. Tree and log files were combined in Logcombiner v.1.7 and results were visualized in Tracer v.1.6 (Rambaut et al. 2018), to evaluate whether the effective sample size (ESS) values were above 200 and to check for parameter convergence. The resulting trees were summarized in a maximum clade credibility tree by TreeAnnotator v.1.7. (Bouckaert et al. 2014) with a burnin of 5000 trees for each run.

Second, the subtree formed by the crown group for all *Xylodon australis* s.l. specimens analyzed was selected as a base tree for the next analyses. Five continuous morphological traits usually known as taxonomically informative for Hymenochaetales were selected: basidia length and width, spore length and width, and spore length-width ratio (Parmasto et al. 1987). These traits were measured for the *X. australis* type specimen and for all samples in the subtree. To place the type specimen of *X. australis* into the molecular tree using the continuous morphological traits, the function *locate.yeti* from the R package *phytools* (v0.6.60) was used (Revell 2012). This function adapts the approach proposed by Felsenstein (1973, 1981) to estimate phylogeny from continuous traits using a maximum likelihood framework. To include more than one continuous trait, a phylogenetic principal component analysis is performed first (Revell, 2009). Then, these principal components are used to identify the optimal position of the type specimen in the phylogenetic tree applying the maximum likelihood criterion (see equation 1 in Revell et al. 2014). The method relies on the assumption that the characters have evolved along the tree and that the morphological differences between species are mostly due to inherited genetic differences.

In order to assess the performance of this approach, a randomization test with the original pure molecular subtree was conducted. We ran 100 replicates in which we pruned one tree-tip at random per replicate. Then, the tip was included again in the tree in two ways: randomly located or using the *locate.yeti* function. These two trees were compared with the original molecular tree by computing branch-score (Kuhner & Felsenstein 1994) and quadratic path distances (Steel & Penny 1993) using the R package *phangorn* (Schliep 2011). A lower distance means more similarity of the reconstructed tree with the actual molecular tree. In addition, to test the hypothesis that the inferred ML phylogenetic position of the type specimen through *locate.yeti* function is significantly better than

alternative locations, a likelihood ratio test was conducted by comparing the likelihood score of tree in which type position was constrained, to trees with unconstrained type locations. Simulations of continuous traits were performed on the constrained tree, and then type position was inferred without constraint. These simulations were used to generate a null distribution to check for significance of the likelihood ratio test (Revell et al. 2014).

The same methodology was applied to each specimen from New Zealand (PDD herbarium) for which no molecular data were obtained.

RESULTS

Phylogenetic analyses

A total of 66 new sequences were generated in this study: 35 sequences for ITS region and 31 for LSU. Final alignments including sequences from EMBL/GenBank/DDBJ databases contained 110 ITS sequences for a dataset length of 724 characters and 87 LSU sequences with 987 characters. All new sequences have been deposited in the EMBL/GenBank/DDBJ database and their accession numbers are presented in Tables 1 and S1.

The identity of all samples named under *X. australis* was confirmed by molecular data (Figure S1–S3) and their phylogenetic position among other *Xylodon* species was according to Riebesehl et al. (2019).

Statistical tests of morphological characters

ANOVA on basidia and spore morphology was conducted on eight Australian and twelve Patagonian specimens. Significant differences were found for all measures: basidia length and width, and spore length, width and length/width relation (Table 2; Fig 1). Specimens from Australia showed longer and wider basidia than samples from Patagonia. In the same way, spores of specimens from Australia were longer and wider than those of Patagonian samples. Spore length/width ratio for the Australian lineage was lower than for the Patagonian clade, thus the former has spores narrowly ellipsoid or subcylindric compared to the latter.

Inferring the position of the nomenclatural type of Xylodon australis in the molecular phylogenetic tree

The ultrametric phylogenetic trees of 47 *Xylodon* specimens gave the same topology for the ITS (not shown), LSU (not shown), and ITS + LSU datasets (Fig 2). Effective sample sizes for all parameters were higher than 200. Bayesian Inference analyses showed that

Table 2. Statistical tests of morphological characters. ANOVA on basidia and spore morphology.

	F(1, 18)	**P - value**
Basidia length	70.34	< 0.01
Basdia width	23.65	< 0.01
Spore length	30.50	< 0.01
Spore width	208.4	< 0.01
Spore length/width	22.13	< 0.01

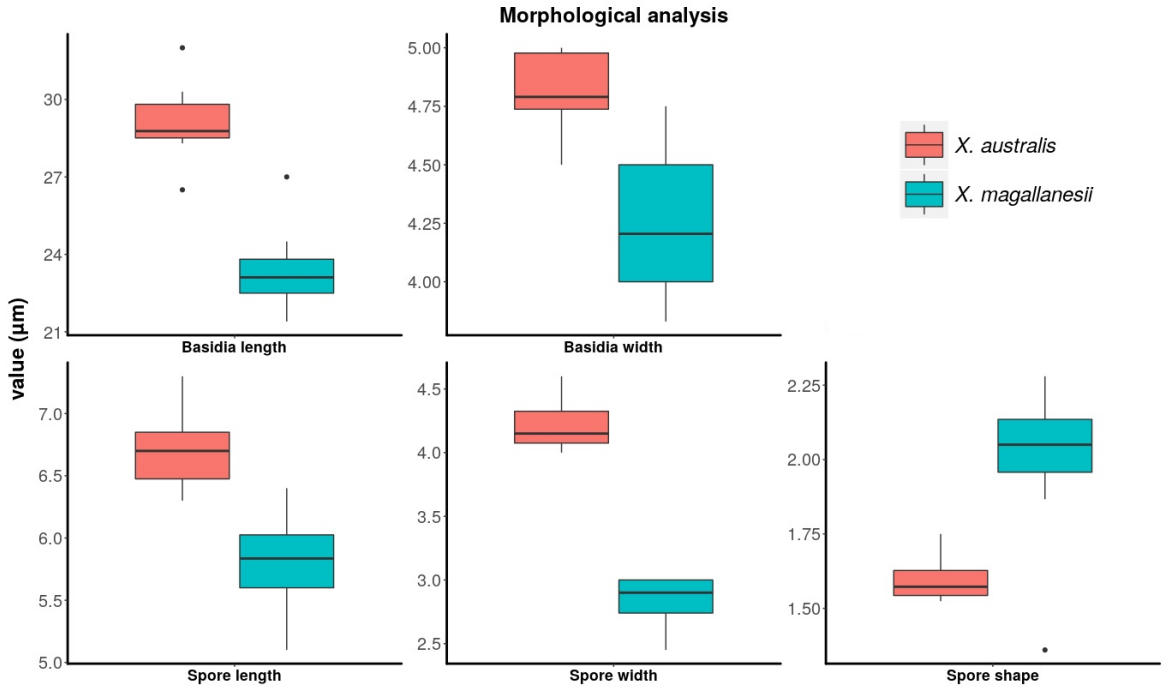

Fig 1. Morphological analyses conducted for basidia length and width, and spore length, width and length/width ratio.

specimens under the *X. australis* name were distributed in two non-directly-related and highly-supported clades. All Australian collections were grouped in one clade, while the other clade included all the Chilean and Argentinean specimens. Three sequences of species *X. lenis* (including from the type specimen) are the sister clade of the Chilean and Argentinean specimens; this relationship has strong support in all datasets (ITS PP=1.0, LSU PP=0.99, and ITS + LSU PP=1). *Xylodon lenis*, *X. australis* s.l. from Patagonia and *X. australis* s.l. from Australia formed the crown clade for all *X. australis* s.l. specimens (ITS + LSU PP=0.97).

The topology of the combined dataset for this clade was used as subtree to infer the phylogenetic position of *X. australis* type specimen using continuous morphological traits (Fig. 3A), as well as to the New Zealand samples from which no sequences were obtained (Figure S4).

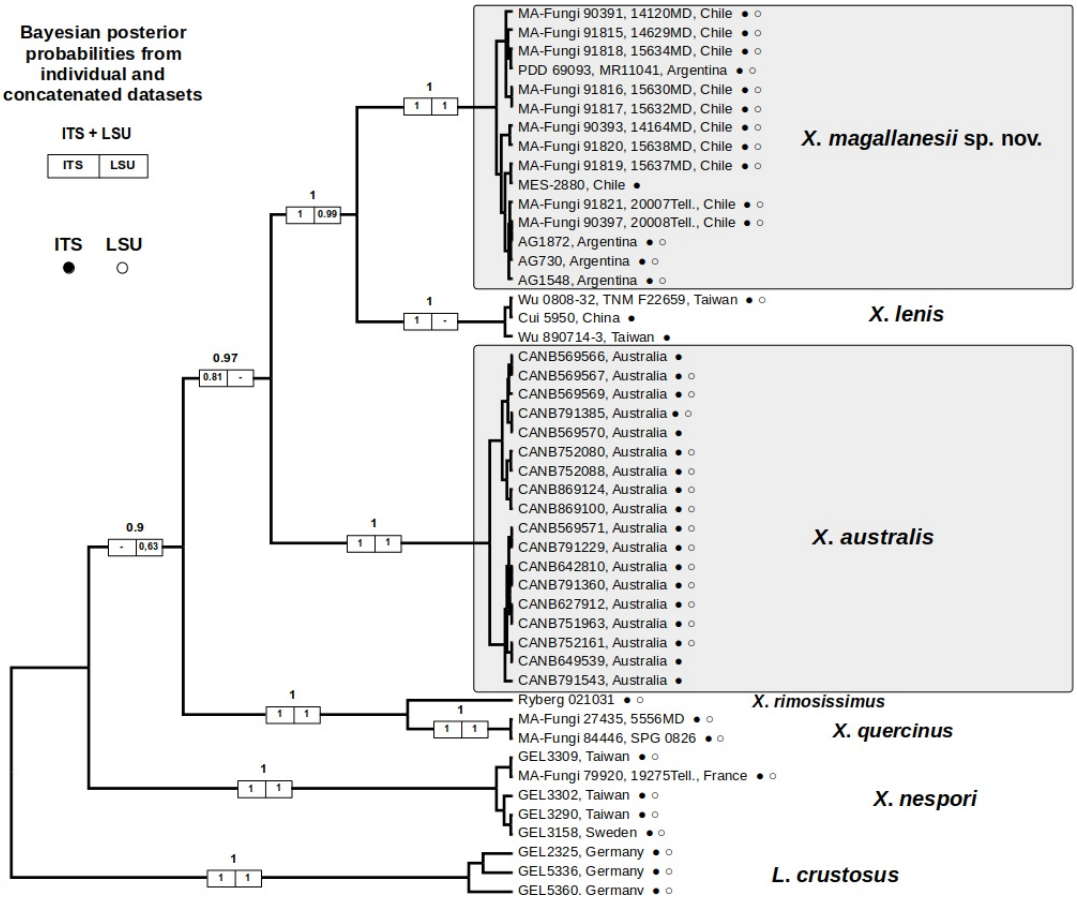

Fig 2. Ultrametric phylogenetic trees to obtain the *Xylodon australis* complex crown group. Topology showed correspond to the bayesian tree for combined ITS + LSU datasets.

The type specimen of *Xylodon australis* was located in the Australian molecular lineage, according to the five continuous morphological traits used, under a maximum likelihood framework using the *locate.yeti* (Fig 3A).

Results of the randomization test to assess the accuracy of the method for our case study are shown in Fig 3B, C. In general, trees reconstructed according to continuous mophological traits, that is, using *locate.yeti*, were more similar to the actual molecular tree than those reconstructed by random tip location (lower values of branch score and

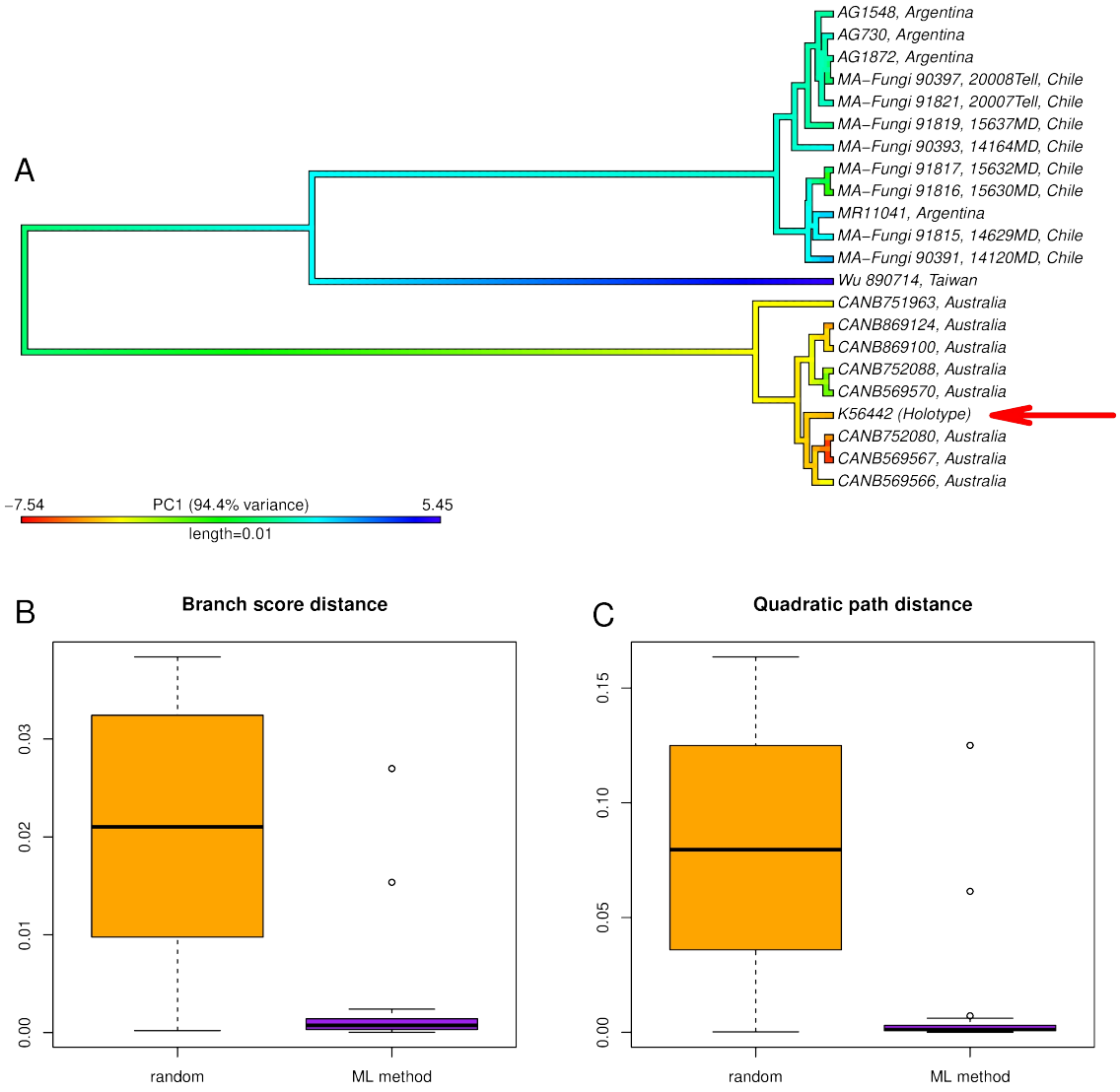

Fig 3. Statistical tests of morphological characters. ANOVA on basidia and spore morphology. Results of analysis to infer the position of the type material of *Xylodon australis* using *locate.yeti* function. A. Position of the type material. B. and C. Branch score and quadratic paths distances from the original molecular tree.

quadratic paths distances, Fig 3B, C). The likelihood ratio test was conducted by constraining the type position to the Patagonian clade (the alternative position to our results). The hypothesis that the type collection of *X. australis* belongs in the Patagonian clade was rejected by our analyses (P-value < 0.01; Table 3).

Table 3. Likelihood ratio test conducted for constrained and unconstrained type position to the Patagonian clade.

Model	log(L)	P-value (compared to unconstrained model)
Unconstrained	-14.27	-
Constrained to Patagonian clade	-35.24	< 0.01

TAXONOMY

Xylodon magallanesii J. Fernández-López, Telleria, M. Dueñas, M. Laguna & M.P. Martín, **sp. nov.** MycoBank MB 834687 (Fig 4)

Etymology: Named after Fernando de Magallanes (1480–1521), the Portuguese explorer who commanded the Spanish expedition to the East Indies from 1519 to 1522, resulting in the first circumnavigation of the Earth, which was completed by Juan Sebastián Elcano.

Diagnosis: Morphology similar to *Xylodon australis*, but differs in having smaller basidia, 21–24 × 4–4.5 µm, and smaller and narrowly ellipsoidal to subcylindrical basidiospores, (5–)5.5–6(–6.5) × 2.5–3(–3.5) µm with Q=2.03.

Type: CHILE, Los Lagos (X Región), Palena, Comuna Hualaihué, Reserva de Huinay, road to Lloncochaigua river, 42º22'38.9"S 72º24'45.8"W, 190 msl, on dead wood, 30 Apr. 2012, M. Dueñas, M.P. Martín & M.T. Telleria, 20008Tell. (holotype, MA-Fungi 90397), ITS and LSUsequences GenBank MT158729 and MT158765.

Description: Basidioma resupinate, effuse; hymenophore sometimes cracked, odontoid or hydnoid, with unequal granules or teeth, 1–4/mm, light pink to dark pink for wet material (4.l.Pink – 5.m.Pink – 6.d.Pink), light orange to deep orange for dry material (52.l.O – 50.s.O – 51.deepO), violet in KOH; margin not clearly differentiated. Hyphal system monomitic; generative hyphae hyaline, thin to thick-walled, with clamps, 3–5 µm in diam. Subicular hyphae interwoven, walls up to 1 µm thick, scarcely branched.

Subhymenial hyphae thinner, branched. Cystidia present: 1) moniliform cystidia scarce, arise from the subiculum, 35–45 × 2.5–5 µm, thin-walled, sometimes with basal clamp; 2) claviform to slightly moniliform cystidia, sometimes with a granulous cap, 25–35(–45) × 3–5 µm, thin-walled, basal clamp. Basidia narrowly clavate, 21–24 × 4–4.5 µm, four sterigmata, with basal clamp. Basidiospores narrowly ellipsoidal to subcylindrical, (5–)5.5–6(–6.5) × 2.5–3(–3.5) µm, hyaline, thin-walled, smooth, usually with several oil drops. L=6.06, W=2.98, Q=2.03 (n=20).

Ecology & Habitat: On dead wood of *Nothofagus betuloides*, *N. dombeyi*, *N. pumilio*, *Amomyrtus luma* and *Drymis winteri*.

Known distribution: Reported from the Patagonian region (southern Chile and southern Argentina).

Additional material examined: ARGENTINA, Río Negro, Parque Nacional Nahuel Huapi, Puerto Blest, road to Los Cántaros, on fallen log of *Nothofagus dombeyi*, 31 Oct. 1995, *M. Rajchenberg* 11041 (PDD 69093); Tierra de Fuego, Ushuaia, Paso Garibaldi, on *Nothofagus pumilio* or *N. betuloides*, 27 Mar. 1998, *A. Greslebin* 1548; ibid., *Nothofagus betuloides*, 10 Nov. 1998, *A. Greslebin* 1872; Tierra de Fuego, Ushuaia, Tolhuin, 3 km East from Hostería Kaikén, on *Nothofagus pumilio*, 4 Nov. 1996, *A. Greslebin* 730. CHILE, Los Lagos (X Región), Palena, Comuna Hualaihué, Reserva de Huinay, "Cementerio de alerces", 42º21'57.9"S 72º24'56.9"W, 30 msl, on *Amomyrtus luma*, 29 Apr. 2012, *M. Dueñas, M.P. Martín & M.T. Telleria*, 14120MD (MA-Fungi 90391); ibid., "Derrumbe Antiguo", 42º22'17.0"S 72º24'12.2"W, 120 msl, on *Nothofagus dombeyi*, 1 May 2012, *M. Dueñas, M.P. Martín & M.T. Telleria*, 14163MD (MA-Fungi 90392); idem, *M. Dueñas, M.P. Martín & M.T. Telleria*, 14164MD (MA-Fungi 90393); ibid., 42º22'01.5"S 72º24'57.8"W, 50 msl, on *Drymis winteri*, 10 May 2013, *M. Dueñas, M.P. Martín & M.T. Telleria*, 14629MD (MA-Fungi 91815); ibid., road to Lloncochaigua river, 42º22'38.9"S 72º24'45.8"W, 190 msl, on dead wood, 4 May 2013, *M. Dueñas, M.P. Martín & M.T. Telleria*, 20007Tell. (MA-Fungi 91821); Los Ríos (XIV Región), Ranco, Comuna de La Unión, Road T-80, 40º13'49.3"S 73º21'38.4"W, 664 msl, on dead wood, 06 Nov. 2017, *M. Dueñas, J. Fernández-López, M.P. Martín, S. Nogal-Prata & M.T. Telleria*, 15630MD (MA-Fungi 91816); idem, *M. Dueñas, J. Fernández-López, M.P. Martín, S. Nogal-Prata & M.T. Telleria*, 15632MD (MA-Fungi 91817); idem, *M. Dueñas, J. Fernández-López, M.P. Martín, S. Nogal-Prata & M.T. Telleria*, 15634MD (MA-Fungi 91818); idem, *M. Dueñas, J. Fernández-López, M.P. Martín, S. Nogal-Prata & M.T. Telleria*, 15637MD (MA-Fungi

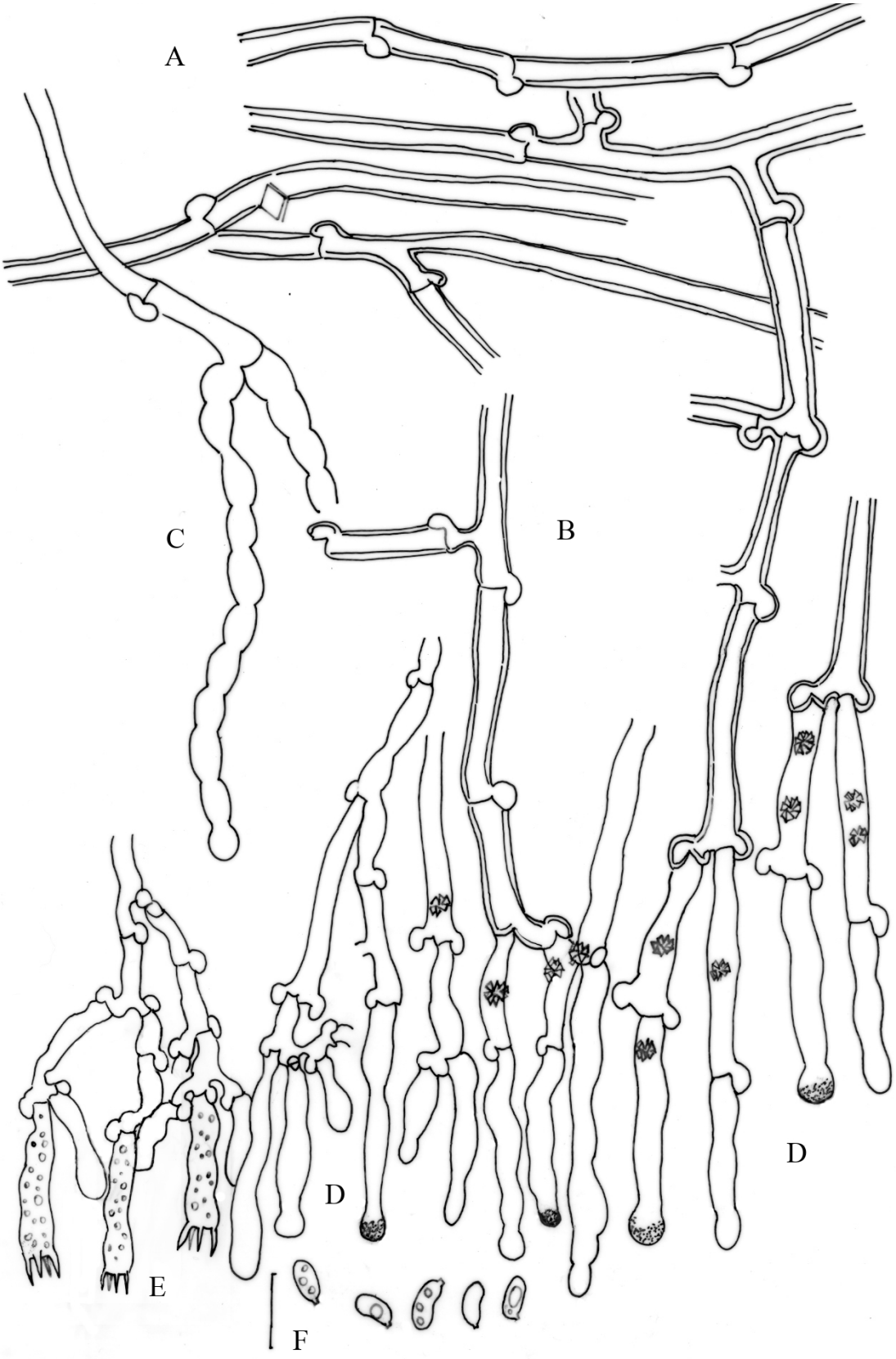

Fig 4. *Xylodon magallanesii*, 20008Tell, MA-Fungi 90397, holotype. A. Subicular hypha. B. Suhnymenial hypha. C. Moniliform cystidia. D. Claviform cystidia. E. Basidia F. Basidiospores. Bar=10 µm. Drawn by Margarita Dueñas.

91819); idem, *M. Dueñas, J. Fernández-López, M.P. Martín, S. Nogal-Prata & M.T. Telleria*, 15638MD (MA-Fungi 91820).

Other material examined (*Xylodon australis*): AUSTRALIA, Australian Capital Territory, "Birrigai", 22 km SW of Capital Hill, Canberra, 35º28'S 148º57'E, 700 msl, in an open paddock, 16 May 1992, *H. Lepp* 818 (CANB 569566); ibid., Orroral to Cotter Hut road, 38 km SW of Capital Hill, Canberra, 35º37'S 148º55'E, 1100 msl, on fibrous bark of live *Eucalyptus* trunk, 12 Jun 1993, *H. Lepp* 964 (CANB 569567); ibid., Tidbinbilla Nature Reserve, 27 km SW of Capital Hill, Canberra, 35º27'S 148º53'E, 800 msl, on a fallen, rotting *Eucalyptus* trunk, 21 Febr 1993, *H. Lepp* 905 (CANB 569570); New South Wales, Southern Tablelands, Morton National Park, near Endrich River, Round mountain, *Eucalyptus* forest, 35º10'12"S 150º09'37"E, 700 msl, on small branch on ground, 26 Jul. 2011, *P. Wellman* 697, det. *H. Lepp* (CANB 869100); ibid., Brindabella National Park, near Canberra. Doctors Flat Road, open *Eucalyptus* forest, 35º13'48"S 148º52'40"E, 887 msl, on *Eucalyptus* log, 20 Dec. 2011, *P. Wellman* 711A (CANB 869124); Queensland, Darling Downs, Girraween National Park, *Eucalyptus* dominated woodland, 3 May 2005 (CANB 751963); ibid., Burnett, Bunya Mountains National Park, rain forest, 6 May 2005 (CANB 752080); ibid., a little below the summit of Mt Kiangarow, 26º49'45"S 151º 33'00"E, 1130 msl, on rotting branch of live tree, 06 May 2005, *H. Lepp* 4827 (CANB 752088). Tasmania, unlocalized, ex herb *M.J. Berkeley* (type K(M) 56442); Flowery Gully, on the underside of a rotting log, 21 Apr. 1992 (CANB 569568); ibid., Gunner's Quoin, Hobart; *Eucalyptus* woodland 28 Apr. 1992 (CANB 569572); NEW ZEALAND, Auckland, Mt. Te Aroha, 600 msl, on *Brachyglottis repanda*, Nov. 1946, *G.H. Cunningham* (PDD 23689); ibid., 900 msl, on *Coriaria arborea*, Nov. 1946, *G.H. Cunningham* (PDD 23691); ibid., Thames, Waiomo Valley, on *Coriaria arborea*, 21 Aug. 1954, *J.M. Dingley* (PDD 23692); ibid., Camel's Back, Coromandel, 800 msl, on *Coriaria arborea*, 25 Oct. 1954, *J.M. Dingley* (PDD 23693); ibid., Little Huia, 200 msl, on *Leptospermum ericoides*, 24 Dec. 1949, *E.E. Chamberlain* (PDD 23696); ibid., Swanson, on *Leptospermum ericoides*, 18 Apr. 1954, *J.M. Dingley* (PDD 23698); ibid., Whakarewarewa, Rotorua, on *Eucalyptus globulus*, 14 Jun. 1950, *J.M. Dingley* (PDD 23699); ibid., Waipous Kauri Forest, on *Leptospermum ericoides*, 30 Sep. 1949, *J.M. Dingley* (PDD 23703); ibid., Huia, on *Leptospermum ericoides*, 17 Jan. 1955, *Mrs. E.E. Chamberlain* (PDD 23704); ibid., Piha, Glen Esh Valley, on *Leptospermum ericoides*, 31 March. 1956, *J.M. Dingley* (PDD 23705); Westland, Fox Glacier Road, 600 msl, on *Coriaria arborea*, Nov. 1946, *G.H. Cunningham* (PDD 23694).

Other material examined (*Xylodon lenis*): TAIWAN, Kaohsiung: Liukuei Hsiang, Shanping, 770 msl, on fallen twig of angiosperm, 14 Jul. 1989, *Sheng Hua Wu* 890714-3 (isotype).

Notes: Although *X. magallanesii* is closely related to *X. lenis* based on ITS and LSU sequences, it is morphologically closer to *X. australis*. In addition to their smaller basidia and basidiospores, *Xylodon magallanesii* can be distinguished from *X. australis* by their smaller claviform cystidia with a granulose cap, up to 45 µm length. There are clear morphological differences between *X. lenis* and *X. magallanesii*: *X. lenis* presents smaller basidia (16–21 µm length) and smaller and broadly ellipsoidal spores (4.2–5 × 3–3.5 µm). A shared character among the three species is the hymenophore color change after the application of KOH, turning from orange to violet.

DISCUSSION

The exhaustive study carried out by Greslebin et al. (2000) was not enough to consider *Xylodon australis* and *Xylodon magallanesii* as two different species due to the lack of evidence in addition to morphology. However, these authors pointed toward a speciation process due to the differences found in spore size and shape of samples from each area. Our phylogenetic analyses not only confirmed the identity of two species under the *X. australis* name, but also revealed the relation of *Xylodon lenis* as the sister species of *Xylodon magallanesii* (Fig 2).

Our microscopic studies agree with Greslebin et al. (2000), and showed statistically significant differences in basidia and spore size and shape between *X. australis* and *X. magallanesii* (Fig 1). Spores of *X. magallanesii* were in general smaller, as were their basidia. This correlation between spore and basidia size has been traditionally reported (Corner 1948; Meerts 1999) and, therefore, our results could be expected. However, spore shape can be related to more complex responses, including dispersal abilities, bioclimatic fitness, or life history characteristics (Kauserud et al. 2008; Fernández-López et al. 2019), so a specific study should be carried out to explain differences in basidiospore shape between *X. australis* and *X. magallanesii*. No molecular data were obtainable from New Zealand samples studied under the *X. australis* name; for this reason, these specimens were not included in our statistical analysis in order to compare only those clades confirmed by molecular data. Taking into account our results from *X. magallanesii*, and despite the fact that our ML analyses using the *locate.yeti* function included all New

Zealand samples in the Australian clade (Fig S4), future analyses should be carried out in order to confirm the identity of the *X. australis* samples from New Zealand. In contrast to the type specimen of *X. australis*, samples from PDD addressed in this study are available for destructive sampling, and therefore new approaches for DNA extraction could be successful.

The inferred position by *locate.yeti* function for the type of *X. australis* in the molecular tree arranged with Australian samples, supported the designation of *X. magallanesii* samples from Chile and Argentina as the new species (Fig 3A). Our analysis resulted in a high performance in the validation test, and was able to correctly locate the pruned molecular taxon better than randomness for our study group (Fig 3B, C). These results may be due to choosing morphological traits to infer the phylogenetic position, which are known to be taxonomically informative in differentiating closely related species in *Xylodon* (Fernández-López et al. 2018). Since the performance of the method is known to increase with the amount of data (Revell et al. 2014), the quality of morphological traits could be a key factor when the number of characters is small or the number of taxa in the molecular tree is less than 20, as in our case. In addition, the likelihood ratio test rejected the alternative position proposed for the *Xylodon australis* type in the *X. magallanesii* clade. Therefore, its position with the Australian samples is strongly supported. These results are also in accordance with geographic evidence, since the type specimen of *Xylodon australis* was collected in Tasmania and therefore their connection with specimens from Australia was expected.

The close phylogenetic relation between *Xylodon australis*, *X. lenis* and *X. magallanesii* is reported in our study for the first time. *Xylodon lenis* was described as *Hyphodontia mollis* by Wu in 1990 from Taiwan. Though the *Xyldon australis* characteristic color change with the application of KOH was not described for *X. lenis*, Wu (1990) highlighted the presence of granular material over the hyphal system that dissolves in KOH, a character also shown by *X. australis* and *X. magallanesii*. In an additional macromorphological inspection conducted on an *Xylodon lenis* isotype (Wu 890714-3) for this study, we could observe color change from orange toward violet after the application of KOH. Therefore, as in other fungal groups (Singer 1962; Martín 1996), this character emerges as a useful trait for taxonomic classification when closely related species are compared. Another morphological character that points to a relation among these three species is the cracked hymenial surface, described in several studies (Wu 1990; Greslebin

et al. 2000) and also shown by *Xylodon magallanesii*.

From a biogeographical point of view, these phylogenetic relations are a challenge due to the distribution pattern of the species. The spatial structure inside each species remains congruent and a geographic isolation between them is shown in our results (Fig 2). The sister relation between *Xylodon magallanesii* from Patagonia and *X. lenis* from China and Taiwan could be explained as an example of long distance dispersion (Golan & Pringle, 2017). This ability has been confirmed for many fungi, even for the same South America–Asia–Australia pattern in some cases, such as for the *Ganoderma applanatum-australe* species complex (Moncalvo & Buchanan 2008). Other possible explanations for the disjunct distribution of sister species may be due to incomplete taxon sampling, or to the extinction of lineages that had linked these species in the past.

Epitypification and neotypification have been proposed as possible solutions to address taxonomic confusion in those cases where: type specimens are damaged, characters used for species identification are not manifest, or the material is not available (Ariyawansa et al. 2014). However, these solutions should be applied cautiously, since they have many other associated risks (Ariyawansa et al. 2014). Some other authors (Dayarathne et al. 2016) have argued that, although the best possible solution would be to examine the type material, one option could be to describe new and well documented taxa and to ignore old species names, but other problems such as taxonomic inflation, could arise with this practice (Isaac et al. 2004).

Our study shows how new methodological approaches can help to solve old taxonomic problems that have became more evident during the DNA era. The possibility to place a type collection into a molecular tree, using phenotypic traits, increases the value of herbaria and museum collections. This is especially important in groups such as *Xylodon*, in which new species and combinations are being proposed every year (Riebesehl et al. 2019), and taxonomy is quickly changing (Riebesehl & Langer 2017). The study of type materials is essential to avoid bad taxonomy that could lead to important ecological and economic losses (Bortolus 2008).

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Table S1. Specimens and sequences included for the phylogenetic analyses. Data of voucher, and publication if available. New sequences obtained in this study in bold. (-)=Sequences not obtained or nor available in GenBank.

Species	Specimen voucher	GenBank Acc. Nº.		Reference
		ITS	LSU	
Xylodon apacherensis (Gilb. & Canf.) Hjorstam & Ryvarden	Canfield 180, Holotype	KY081800	-	Riebesehl & Langer (2017)
X. asperus (Fr.) Hjorstam & Ryvarden	KH Nilsson s.n.	DQ873606	DQ873607	Larsson et al. 2006
	Larsson 8530 (GB)	-	AY586675	Larsson et al. 2004
	UC2023169	KP814365	-	Riebesehl & Langer 2017
X. astrocystidiatus (Yurchenko & Sheng H. Wu) Riebesehl, Yurchenko & E. Langer	Wu 9211-71	JN129972	JN129973	Yurchenko & Wu 2014
X. attenuatus Spirin & Viner	Spirin 8775, Holotype	MH324476	-	Viner et al. 2018
X. australis (Berk.) Hjorstam & Ryvarden	CANB569566	MT158734	-	**This study**
	CANB569567	MT158703	MT158739	**This study**
	CANB569569	MT158704	MT158740	**This study**
	CANB569570	MT158735	-	**This study**
	CANB569571	MT158705	MT158741	**This study**
	CANB627912	MT158706	MT158742	**This study**
	CANB642810	MT158707	MT158743	**This study**
	CANB649539	MT158736	-	**This study**
	CANB751963	MT158708	MT158744	**This study**
	CANB752080	MT158709	MT158745	**This study**
	CANB752088	MT158710	MT158746	**This study**
	CANB752161	MT158711	MT158747	**This study**
	CANB791229	MT158712	MT158748	**This study**
	CANB791360	MT158713	MT158749	**This study**
	CANB791385	MT158714	MT158750	**This study**
	CANB791543	MT158737	-	**This study**
	CANB869100	MT158715	MT158751	**This study**
	CANB869124	MT158716	MT158752	**This study**
X. borealis (Kotir. & Saaren.) Hjorstam & Ryvarden	JS26064	-	AY586677	Larsson et al. 2004
	Spirin 9416	MH317760	MH638259	Viner et al. 2018
	UC2022850	KP814307	-	Rosenthal et al. 2017
X. brevisetus (P. Karst.) Hjorstam & Ryvarden	JS17863	-	AY586676	Larsson et al. 2004
	Larsson 12386 (GB)	DQ873612	DQ873612	Riebesehl et al. 2019
	UC2023199	KP814485	-	Unpublished
X. bubalinus (Ming Wang, Yuan Y. Chen & B.K. Cui) C.C. Chen & Sheng H. Wu	CL Zhao 184	MG231628	-	Unpublished
	Cui 6834	KY290981	-	Wang & Cheng 2017
	Cui 12887	KY290982	-	Wang & Cheng 2017
	Cui 1288, Holotype	KY290983	-	Wang & Cheng 2017
X. chinensis (C.C. Chen & Sheng H. Wu) C.C.Chen & Sheng H. Wu	Wu 1307-42	KX857802	-	Chen et al. 2017
	Wu 1407.105, Holotype	KX857804	KX857811	Chen et al. 2017
X. crystalliger Viner	KUN2312, Holotype	MH324477	-	Viner et al. 2018
X. cystidiatus (A. David & Rajchenb.) Riebesehl & Langer	FR-0249200	MH880195	MH884896	Riebesehl et al. 2019
X. detriticus (Bourdot) K.H. Larss.,Viner & Spirin	UC203108	KP814412	-	Rosenthal et al. 2017
	Zíbarová 30.10.17	MH320793	MH651372	Viner et al. 2018
X. dimiticus (Jia J. Chen & LW. Zhou) Riebesehl & Langer	Dai 15321	KT989969	-	Chen et al. (2016)
X. exilis Yurchenko, Riebesehl & E. Larger	MSK-F 7381	MH880196	MH884897	Riebesehl et al. 2019
	MSK-F 7431	-	-	Riebesehl et al. 2019
	TUB-FO 42450	MH880197	-	Riebesehl et al. 2019
	TUB-FO 42565, Holotype	MH880198	MH884898	Riebesehl et al. 2019

Table S1 (cont.).

Species	Specimen voucher	GenBank Acc. Nº.		Reference
		ITS	LSU	
X. filicinus Yurchenko & Riebesehl	MSK-F12869, Holotype	MH880199	MH884899	Riebesehl et al. 2019
	MSK-F 12870	MH880200	MH884900	Riebesehl et al. 2019
X. flaviporus (Berk. & M.A. Curtis ex Cooke) Riebesehl & E. Langer	FR-0249797	MH880201	MH884901	Riebesehl et al. 2019
	FUG 1053	AF145575	-	Paulus et al. 2000
	ICMP 13836	AF145585	-	Paulus et al. 2000
	GEL3462 (KAS)	MH880202	-	Riebesehl et al. 2019
	GEL5047 (KAS)	MH880203	-	Riebesehl et al. 2019
	KUC20130808-17	-	KJ668314	Jang et al. 2014
X. follis Riebesehl, Yurchenko & langer	FR-0249814, Holotype	MH880204	MH884902	Riebesehl et al. 2019
X. hastifer (Hjorstam & Ryvarden) Hjorstam & Ryvarden	Ryvarden 19767, Holotype	KY081801	-	Riebesehl & Langer 2017
X. heterocystidiatus (H.X. Xiong, Y.C. Dai & Seng H. Wu) Riebesehl, Yurchenko & E. langer	Wu 9209-27	JX175045	KX857821	Yurchenko & Wu 2014; Chen et al. 2017
X. hyphodontinus (Hjorstam & Ryvarden) Riebesehl,Yurchenko & G. Gruhn	GEL9222 (KAS)	MH880205	MH884903	Riebesehl et al. 2019
	LIP GG-GUY13-044	MH880206	MH884904	Riebesehl et al. 2019
	LIP GG-MAR12-238	MH880207	MH884905	Riebesehl et al. 2019
	LIP GG-MAR15-127	MH880208	MH884906	Riebesehl et al. 2019
X. lenis Hjorstam & Ryvarden	Wu 0808-32	JX175043	KX857820	Yurchenko & Wu 2014
	Wu 890714-3, Isotype	KY081802	-	Riebesehl & Langer 2017
	Cui 5950	KT989972	-	Unpublished
X. magallanesii sp. nov.	14120MD (MA-Fungi 90391)	MT158720	MT158756	**This study**
	14164MD (MA-Fungi 90393)	MT158721	MT158757	**This study**
	14629MD (MA-Fungi 91815)	MT158722	MT158758	**This study**
	15630MD (MA-Fungi 91816)	MT158723	MT158759	**This study**
	15632MD (MA-Fungi 91817)	MT158724	MT158760	**This study**
	15634MD (MA-Fungi 91818)	MT158725	MT158761	**This study**
	15637MD (MA-Fungi 91819)	MT158726	MT158762	**This study**
	15638MD (MA-Fungi 91820)	MT158727	MT158763	**This study**
	20007Tell. (MA-Fungi 91821)	MT158728	MT158764	**This study**
	20008Tell. (MA-Fungi 90397)	MT158729	MT158765	**This study**
	AG 730	MT158730	MT158766	**This study**
	AG 1548	MT158731	MT158767	**This study**
	AG 1872	MT158732	MT158768	**This study**
	CIEFAP-11041 (CFMR)	-	MH884895	Riebesehl et al. 2019
	MES-2880	MH930235	-	Unpublished
	MR 11041 (PDD 69093)	MT158733	MT158769	**This study**
X. mollissimus (L.W. Zhou) C.C. Chen & Sheng H. Wu	LWZ20160318-3, Holotype	KY007517	-	Kan et al. 2017
X. nespori (Bres.) Hjorstam & Ryvarden	GEL3158 (KAS)	DQ340310	DQ340346	Unpublished
	GEL3290 (KAS)	DQ340309	DQ340343	Unpublished
	GEL3302 (KAS)	DQ340308	DQ340344	Unpublished
	GEL3309 (KAS)	DQ340307	DQ340345	Yurchenko & Wu 2014
	JR14 (KAS)	MH880210	-	Riebesehl et al. 2019
	B. Nordon 030915 (GB)	DQ873622	-	Larsson et al. (2006)
	KUC20161012-5	MF774797	-	Unpublished
	19275Tell (MA-Fungi 79920)	MT158717	MT158753	**This study**
X. niemelaei (Sheng H. Wu) Hjorstam & Ryvarden	Dai 15358	KT989973	-	Chen et al. 2016
	FR-0219860	MH880211	-	Riebesehl et al. 2019
	FR-0249174	MH880212	-	Riebesehl et al. 2019
	FR-024178	-	MH884907	Riebesehl et al. 2019
	GC 1508-146	KX857798	-	Chen et al. 2017

Table S1 (cont.).

Species	Specimen voucher	GenBank Acc. Nº.		Reference
		ITS	LSU	
X. niemelaei (Sheng H. Wu) Hjorstam & Ryvarden	GEL4998 (KAS)	EU583422	DQ340348	Unpublished
	Wu 1010-62	-	KX857817	Chen et al. 2017
X. nongravis (Lloyd) C.C. Chen & Sheng H. Wu	CHWC1506-2	KX857800	-	Chen et al. 2017
	GC1412-22	KX857801	KX857818	Chen et al. 2017
	Spirin 5763	MH324469	MH656724	Viner et al. 2018
X. nothofagi (G. Cunn.) Hjorstam & Ryvarden	PDD 91630	GQ411524	-	Fukami et al. 2010
X. ovisporus (Corner) Riebesehl & E. Langer	GEL3493 (KAS)	EU583421	-	Unpublished
	ICMP 13835	-	MH260063	Fernández-López et al. (2018)
	KUC20130725-29	KJ668513	KJ668365	Jang et al. 2016
X. paradoxus (Schrad.) Chevall.	FCUG 1517	AF145572	-	Paulus et al 2000
	FCUG 2425	AF145571	-	Paulus et al 2000
	GEL2511 (KAS)	-	AF518647	Hibbett & Binder 2002
	JR06 (KAS)	MH880219	-	Riebesehl et al. 2019
	JR28 (KAS)	-	MH884908	Riebesehl et al. 2019
	Miettinen 7978	FN907912	FN907912	Miettinen & Larsson 2011
X. pruinosus (Bres.) Spirin & Viner	Spirin 2877	MH332700	-	Viner et al. 2018
	UC2023108	KP814412	-	Rosenthal et al. 2017
X. pseudolanatus Nakasone, Yurchenko & Riebesehl	FP-150922 (CFMR), Holotype	MH880220	MH884909	Riebesehl et al. 2019
X. pseudotropicus (C.L. Zhao, B.K. Cui & Y.C. Dai) Riebesehl, Yurchenko & E. Langer	Dai 10768, Holotype	KF917543	-	Zhao et al. 2014
X. quercinus (Pers.) Gray	5556MD (MA-Fungi 27435)	MT158718	MT158754	**This study**
	Kotiranta 27060	MH320792	-	Viner et al. 2018
	Larsson 11076	KT361633	AY586678	Ariyawansa et al. 2015; Larsson et al. 2004
	Miettinen 15050.1 (H 6013352)	KT361632	-	Ariyawansa et al. 2015
	SPG 0826 (MA-Fungi 84446)	MT158719	MT158755	**This study**
X. raduloides Riebesehl & E. Langer	Dai 12631	KT203307	KT203328	unpublished
	FCUG 2239	-	AF141613	Parmasto & Hallenberg 2000
	ICMP 13833	AF145580	-	Paulus et al 2000
	JR 26 (KAS)	MH880225	MH884910	Riebesehl et al. 2019
	LR 18813	MH880226	MH884911	Riebesehl et al. 2019
X. ramicida Spirin & Miettinen	Spirin 7664, Holotype	KT361634	-	Ariyawansa et al. 2015
X. reticulatus (C.C. Chen & Sheng H. Wu) C.C. Chen & Sheng H. Wu	GC 1512-1	KX857808	KX857813	Chen et al. 2017
	KUC20160721B-26	MF774798	-	Kwon et. al. 2018
	Wu 1109-178, Holotype	KX857805	-	Chen et al. 2017
X. rhizomorphus (C.L. Zhao, B.K. Cui & Y.C. Dai) Riebesehl, Yurchenko & E. Langer	Dai 12354	KF917544	-	Zhao et al. 2014
	Dai 12367, Holotype	KF917545	-	Zhao et al. 2014
	Dai 12389	KF917546	-	Zhao et al. 2014
X. rimosissimus (Peck) Hjorstam & Ryvarden	DLL2011-081 (CFMR)	KJ140600	-	Brazze et al. 2014
	Ryberg 021031 (GB)	DQ873627	DQ873628	Larsson et al. 2006
X. serpentiformis (Langer) Hjorstam & Ryvarden	GEL2668 (KAS)	MH880227	-	Riebesehl et al. 2019
	FO 40675 (TUB)	MH880228	-	Riebesehl et al. 2019
	FO 40985 (TUB)	-	MH884912	Riebesehl et al. 2019
	FO 42688 (TUB)	MH880229	MH884913	Riebesehl et al. 2019
X. sp. 1	Dai 15321	KT989969	-	Chen et al. 2016
X. spathulatus (Schrad.) Kuntze	GEL2960 (KAS)	KY081803	-	Riebesehl & Langer 2017
	Larsson 7085 (GB)	KY081804	-	Riebesehl & Langer 2017
	F 12931 (MSK)	MH880231	MH884914	Riebesehl et al. 2019
	MMS7224 (KAS)	MH880230	-	Riebesehl et al. 2019

Table S1 (cont.).

Species	Specimen voucher	GenBank Acc. Nº.		Reference
		ITS	LSU	
X. subclavatus (Yurchenko, H.X. Xiong & Sheng H. Wu) Riebesehl, Yurchenko & E. Langer	FO 42167 (TUB)	MH880232	-	Riebesehl et al. 2019
X. subflaviporus C.C. Chen & Sheng H. Whu	GEL 3466 (KAS)	MH880253	-	Riebesehl et al. 2019
	Wu 0809-76	KX857803	KX857815	Chen et al. 2017
X. subtropicus (C.C. Chen & Sheng H. Wu) C.C. Chen & Sheng H. Wu	Wu 1508-2	KX857806	KX857812	Chen et al. 2017
	Wu 9806-105, Holotype	KX857807	KX857809	Chen et al. 2017
X. ussuriensis Viner	KUN1989, Holotype	MH324468	-	Viner et al. 2018
X. verecundus (G. Cunn.) Yurchencho & Riebesehl	Larsson 1221 (GB)	DQ873642	-	Larsson et al. 2006
Outgroup				
Lyomyces crustosus (Pers.) P. Karst.	GEL2325 (KAS)	DQ340313	DQ340354	Unpublished
	GEL5360 (KAS)	DQ340315	DQ340355	Unpublished
	GEL5336 (KAS)	DQ340314	DQ340356	Unpublished

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Fig S1. Topology of LSU tree obtained by Bayesian inference (GTR + I + G model). Three sequences of *Lyomyces crustosus* were included as outgroup. Parsimony bootstrap support (≥ 50%), and posterior probability are indicated above branches. Newly generated sequences are indicated in bold. Data from sequences retrieved from EMBL Nucleotide Database (Cochrane et al. 2013) are included in Table S1.

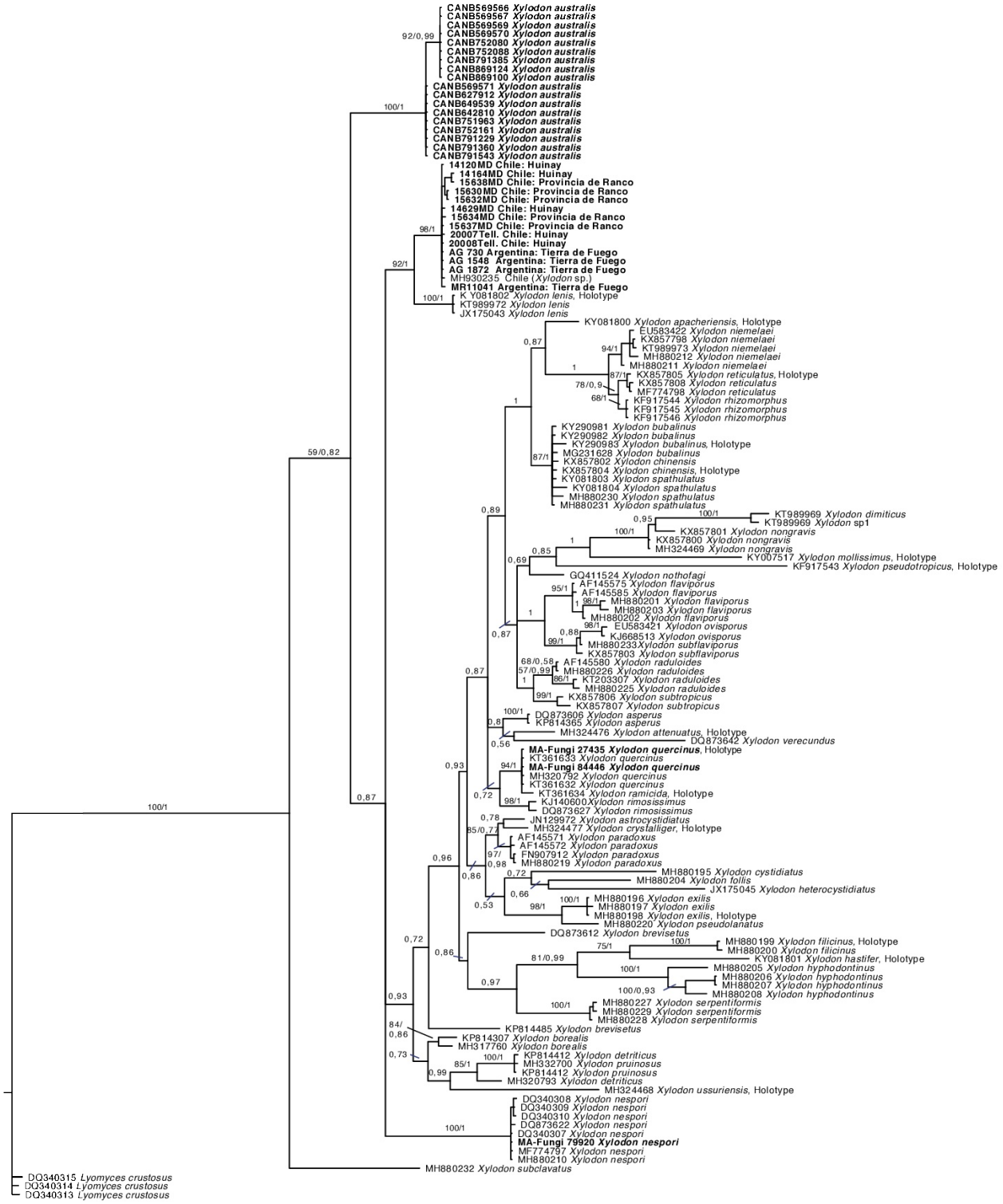

Fig S2. Topology of ITS tree obtained by Bayesian inference (GTR + I + G model). Three sequences of *Lyomyces crustosus* were included as outgroup. Parsimony bootstrap support (≥ 50%), and posterior probability are indicated above branches. Newly generated sequences are indicated in bold. Data from sequences retrieved from EMBL Nucleotide Database (Cochrane et al. 2013) are included in Table S1.

Fig S3. Topology of ITS + LSU tree obtained by Bayesian inference (GTR + I + G model to each marker). Three sequences of *Lyomyces crustosus* were included as outgroup. Parsimony bootstrap support (≥ 50%), and posterior probability are indicated above branches. Newly generated sequences are indicated in bold. Data from sequences retrieved from EMBL Nucleotide Database (Cochrane et al. 2013) are included in Table 1.

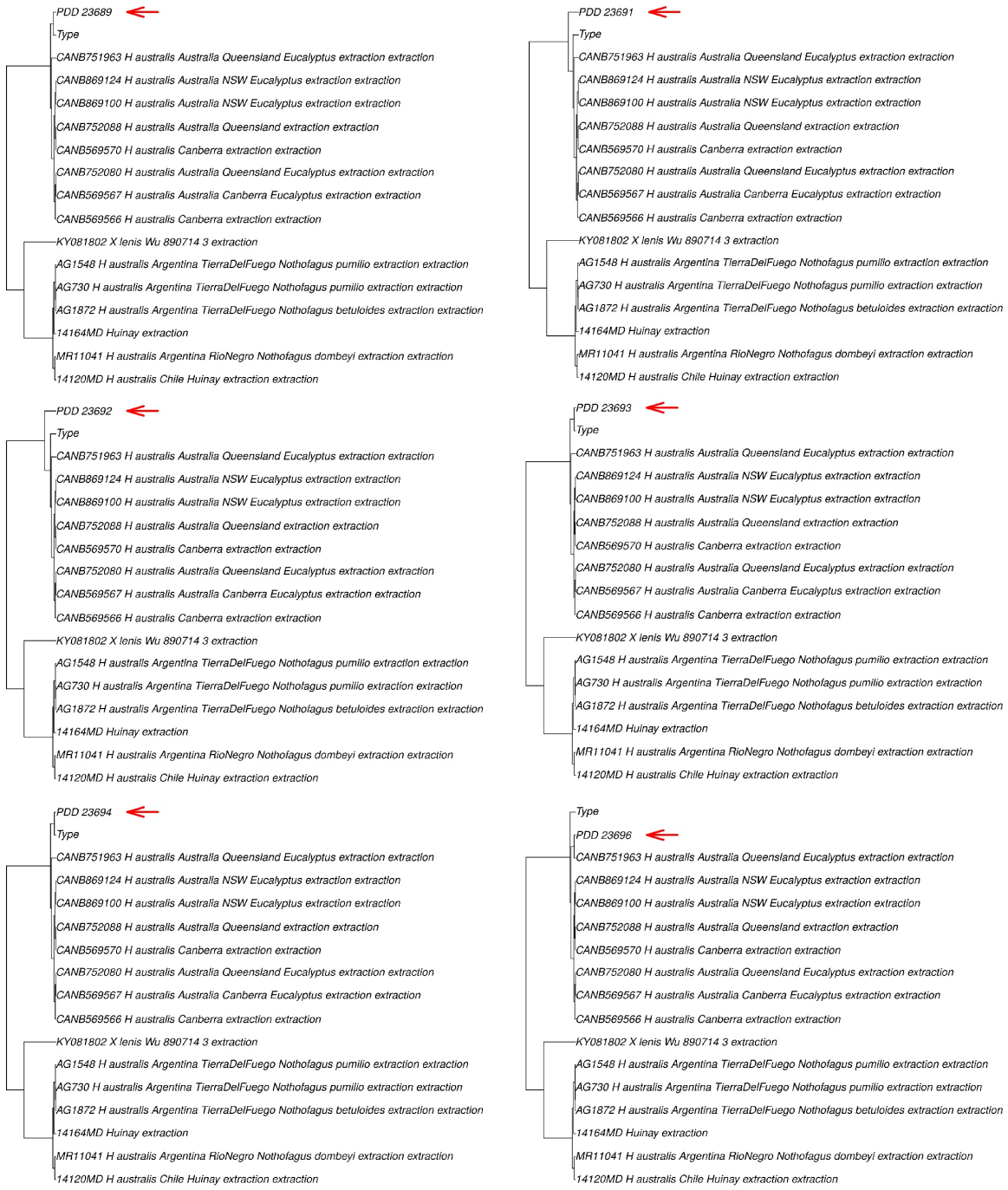

Fig S4. Infered position in phylogenetic tree based on morphological data for New Zealand specimens

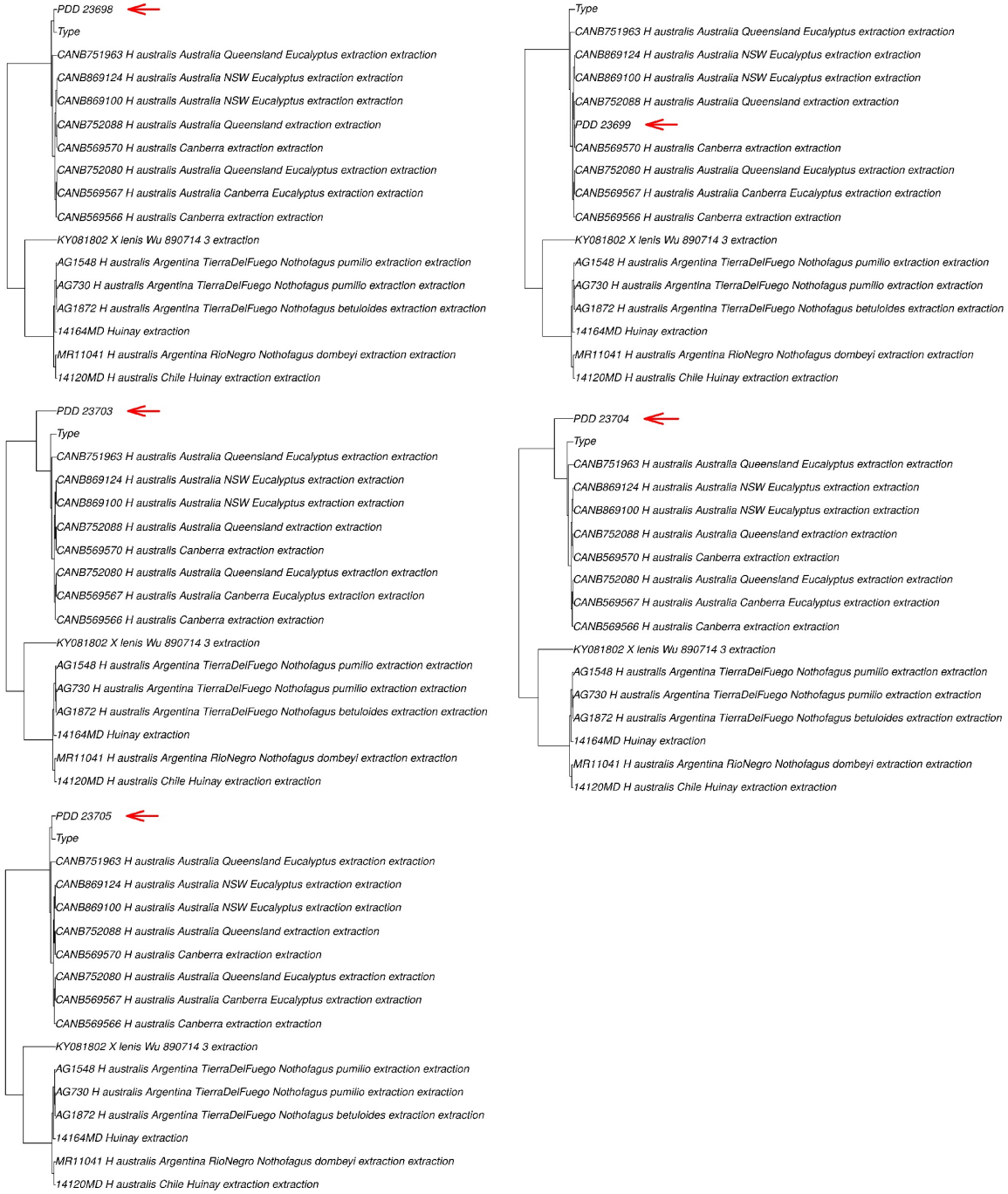

Fig S4 (cont.). Infered position in phylogenetic tree based on morphological data for New Zealand specimens

Chapter 4

Multilocus phylogeny reveals taxonomic misidentification of the *Schizopora paradoxa* (KUC8140) representative genome

Javier Fernández-López, María P. Martín, Margarita Dueñas, M. Teresa Telleria

ABSTRACT

Schizopora paradoxa, current name *Xylodon paradoxus*, is a white-rot fungus with certain useful biotechnological properties. The representative genome of *Schizopora paradoxa* strain KUC8140 was published in 2015 as part of the 1000 Fungal Genomes Project. Multilocus phylogenetic analyses, based on three nuclear regions (ITS, LSU and rpb2), confirmed a misidentification of *S. paradoxa* strain KUC8140 which must be identified as *Xylodon ovisporus*. This wrong identification explains the unexpected geographical distribution of *S. paradoxa*, since this species has a European distribution, whereas the strain KUC8140 was recorded from Korea, Eastern Asia.

INTRODUCTION

The genus *Schizopora* Velen., currently synonymous with *Xylodon* (Pers.) Fr. (Riebesehl & Langer 2017) includes white-rot fungi that play an important role in ecosystem processes as wood decomposer. The description and identification of *Xylodon* (=*Schizopora*) species based on morphological characters has led to inaccuracies due to the lack of clear diagnostic characters, and it has been assumed that many *Xylodon* species have a worldwide distribution (Paulus et al. 2000). However, during the last decade it has been pointed out that fungal cosmopolitanism could be the result of the application of a morphological species recognition criterion and not the result of an actual biogeographical pattern (Taylor et al. 2006). Moreover, phylogenetic analyses have revealed an undescribed species diversity masked by the morphological species recognition approach (Taylor et al. 2000).

The representative genome of *Schizopora paradoxa* strain KUC8140, current name *Xylodon paradoxus* (Schrad.) Chevall., was sequenced in 2015 as part of the 1000 Fungal Genomes Project (http://jgi.doe.gov/fungi) (Min et al. 2015); this strain was collected from an oak forest in Korea. Usually *X. paradoxus* has been associated with late stages of wood decay, mainly in deciduous forests, and shows useful biotechnological properties for bioremediation, such as tolerance to heavy metals or dye decolorizing activity (Lee et al. 2014). It has been recorded around the world; however, available genetic data point to a European distribution (Paulus et al. 2000). In the framework of a broader study of *Xylodon* through molecular approaches, the taxonomic identity of the strain KUC8140 was assessed.

MATERIALS AND METHODS

In order to infer the taxonomic position of the strain KUC8140, phylogenetic relationships of six *Xylodon* species were addressed. DNA extractions for specimens of *X. paradoxus*, *X. quercinus* (Pers.) Gray, *X. nothofagi* (G. Cunn.) Hjorstam & Ryvarden, *X. raduloides* Riebesehl & E. Langer, *X. flaviporus* (Ber. & M.A. Curtis ex Cooke) Riebesehl & E. Langer, and *X. ovisporus* (Corner) Riebesehl & E. Langer were performed from herbaria and culture collections (Table 1). Three specimens of the sister genus *Lyomyces* P. Karst. were included as outgroup in the phylogenetic analyses (Table 1).

Table 1. Specimen information, GenBank accession numbers and genome BLAST searches (ID), used in this study. New sequences generated in this study are indicated in bold. n.d.: no data.

Species	Specimen voucher	Country	GenBank accession number		
			ITS	LSU	*rpb2*
Lyomyces crustosus (Pers.) P. Karst.					
	HHB 10401	USA	**MH260068**	**MH260061**	**MH259316**
	HHB 13100	USA	**MH260069**	**MH260062**	**MH259317**
	UC 2022841	USA	KP814310	-	-
Xylodon flaviporus (Berk. & M.A. Curtis ex Cooke) Riebeschl & E. Langer					
	ICMP 13836	Taiwan	AF145585	-	-
	MA-Fungi 79440, 12094IS	Germany	**MH260071**	**MH260066**	**MH259319**
Xylodon nothofagi (G. Cunn.) Hjorstam & Ryvarden					
	ICMP 13839	New Zealand	AF145582	**MH260064**	**MH259322**
	PDD 91630, BCP 3306	New Zealand	GQ411524	-	-
Xylodon ovisporus (Corner) Riebeschl & E. Langer					
	ICMP 13835	Taiwan	AF145586	**MH260063**	**MH259320**
	ICMP 13837	Taiwan	AF145587	-	-
Xylodon paradoxus (Schrad.) Chevall.					
	FCUG 2425	Russia	AF145577	-	-
	MA-Fungi 70444, 11060MD	France	**MH260070**	**MH260065**	-
	MA-Fungi 81294, 13833MD	France	**MH260072**	-	**MH259318**
Xylodon quercinus (Pers.) Gray					
	H 6013352	Finland	KT361632	-	-
	MA-Fungi 91311, 1JFL	Spain	**MH260073**	**MH260067**	**MH259321**
Xylodon raduloides Riebeschl & E. Langer					
	ICMP 13833	Australia	AF145580	**KY962853**	-
	MA-Fungi 75310, GP2291	Spain	**KY962825**	**KY962864**	**KY967055**
Schizopora paradoxa (Schrad.) Donk					
	KUC8140	Korea	**ID14957398**	**ID14957349**	**ID1495735**

DNA isolation was performed using DNeasy™ Plant Mini Kit (Qiagen, Valencia, California, USA) following the manufacturer's instructions. Three nuclear regions were amplified and sequenced: nuclear ribosomal internal transcribed spacer (ITS) fungal barcoding (Schoch et al. 2012), nuclear large ribosomal subunit (LSU), and second largest subunit of RNA polymerase II (rpb2). Direct Polymerase chain reactions (PCR) were performed to obtain sequences from ITS and LSU with the pair of primers ITS5/ITS4 (White et al. 1990), and LR0R/LR5 (Rehner & Samuels 1994), respectively. Nested-PCRs were done to obtain amplifications of rpb2 fragments, using RPB2-5F/RPB2-7.1R (Liu et al. 1999; Matheny 2005) for the first amplification followed by RPB2-6F/RPB2-7R2 (Matheny et al. 2007), using 1µl of the first PCR as target DNA. Amplifications were done using illustra™ PuReTaq™ Ready-To-Go™ PCR beads (GE Healthcare,

Buckinghamshire, UK) as described in Winka et al. (1998), following thermal cycling conditions in Martín & Winka (2000). Negative controls lacking fungal DNA were run for each experiment to check for contamination. Amplifications were assayed by gel electrophoresis in 2% Pronadisa D-1 Agarose (Lab. Conda, Torrejón de Ardoz, Spain). Amplified DNA fragments were purified from the agarose gel using the Wizard SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI, USA) and sent to Macrogen Korea (Seoul, Korea) for sequencing. Primers used for sequencing were those used for PCR amplifications. Additional searches for the six *Xylodon* species in EMLB/GenBank/DDBJ databases were performed in order to complete the molecular information available for this genus.

Using the BLAST tool from the JGI portal, ITS, LSU and rpb2 sequences were extracted from the KUC8140 strain genome (https://genome.jgi.doe.gov/pages/blast-query.jsf?db=Schpa1). The same regions from *Xylodon paradoxus* specimens FCUG-2425, MA-Fungi 70444, MA-Fungi 81294 were used as reference sequences for BLAST searches respectively (Table 1). For ITS and LSU search settings were fitted (blastn; all databases; Expect=1*10-3; Word size = 11; Filter low complexity regions; Scoring matrix =PAM30; ITS Job ID=14957398; LSU Job ID=14957349). For rpb2, default BLAST settings were used (blastn; assembly database; Expect=1*10-5; Word size=11; Filter low complexity regions; Scoring matrix=BLOSUM62; rpb2 Job ID=14957357). The best scoring sequence from *Schizopora paradoxa* KUC8140 strain genome for each region was extracted and downloaded.

Raw sequence data were processed and assembled with Geneious version 9.0.2. (Kearse et al. 2012). Two individual datasets, ITS-LSU concatenated and rpb2, were created to compare the KUC8140 strain with other *Xylodon* species. The combination of novel, GenBank and KUC8140 sequences for each dataset were aligned in Geneious 9.0.2 with the MAFFT nucleotide sequence alignment function (Katoh & Standley 2013). The automatic alignments were reviewed manually through Geneious 9.0.2.

Phylogenetic tree estimation for each alignment was performed using Maximum Likelihood (ML) and Bayesian inference (BI). ML and bootstrapping analyses were conducted in RAxML (Stamatakis 2006), using default parameters established in the CIPRES web portal (http://www.phylo.org/portal2/; Miller et al. 2010), and calculating bootstrap statistics from 1000 replicates. Bayesian inference analyses were implemented in BEAST v2.4.3 (Drummond & Rambaut, 2007). Site model partition was selected using jModelTest2 (Darriba et al. 2012) and defined using BEAUti v2.4.3 interface. HKY and

GTR substitution models were selected for ITS+LSU and rpb2 alignments respectively as the closest available in BEAST from the results obtained in jModelTest2. We used relative timing with an uncorrelated lognormal relaxed clock by calibrating the tree with a value of 1 in the root for the *Xylodon* clade. Birth Death model was used as a tree prior. One MCMC run was specified for 50 million generations, sampling every 5000th generation. Results were visualized in Tracer v.1.6 (Rambaut et al. 2018), to evaluate whether the effective sample size (ESS) values were above 200. The trees obtained were summarized in a maximum clade credibility tree by TreeAnnotator v.1.7. with a burn-in of 5,000.

RESULTS AND DISCUSSION

The ITS+LSU dataset was 1193 characters long (ITS=594; LSU=599), and rpb2 dataset was 647 characters long. The results of phylogenetic analyses of ITS+LSU and rpb2 datasets are summarized in Fig 1, using *phytools* R package (Revell 2012). Each phylogram represents the best tree produced from the RAxML analysis. All effective sample sizes from BEAST analyses were higher than 200 for all parameters. Those clades with a Maximum likelihood bootstrap (MLB) percentages ≥ 75% and a Bayesian posterior probabilities (BPP) ≥ 0.99 are marked with empty circles in Fig 1. Remaining support values are represented above branches (MLB/BPP); specimen vouchers and species names are provided on the tip labels.

Our phylogenetic analyses confirmed the misidentification of *Schizopora paradoxa* strain KUC8140, since sequences of this strain grouped in the *Xylodon ovisporus* clade, showing a different evolutionary history from *X. paradoxus*. Therefore, *Schizopora paradoxa* strain KUC8140, from Korea, must be identified as *Xylodon ovisporus*, reported from Asia and West Pacific areas (Wu 2000, Hattori 2003). The new identity of the strain KUC8140 is also supported by geographical data, since *S. paradoxa* has a European distribution. This rectification helps to explain the biogeographical patterns of *Xylodon* and sustains the idea that “not everything is everywhere” also for wood-decay fungi (Lumbsch et al. 2008).

According to our phylogenetic analyses *Xylodon ovisporus* is the sister species of *X. flaviporus*, and morphological characters confirm this relationships. The species can be discriminated by the spore size, shorter in the first one (Hattori 2003). This example accords with studies that warn about misidentifications or mislabeled vouchers in public sequence databases (Bidartondo 2008). It has been estimated that around 20% of DNA

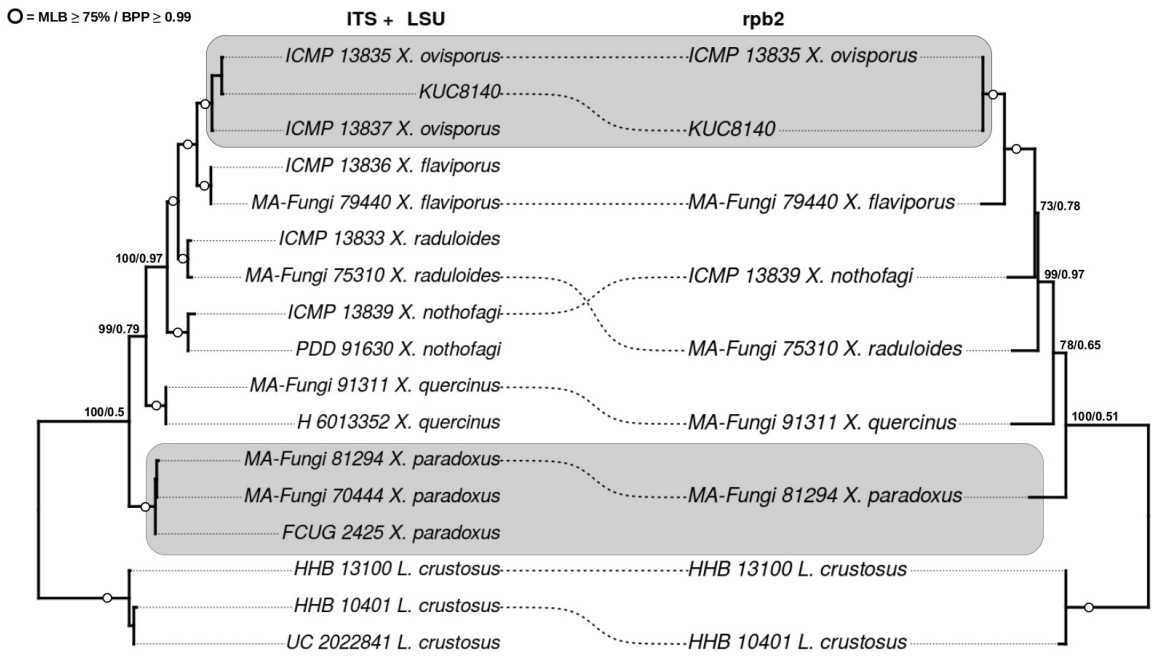

Fig 1. Maximum likelihood trees for ITS+LSU (left) and rpb2 (right) regions of *Xylodon* species. In order to assess genealogical concordance, dotted lines link the position of the same specimen in both trees. Grey boxes indicate the position of KUC8140 strain with *Xylodon ovisporus*, and the position of *X. paradoxus*. Numbers over branches are maximum likelihood bootstrap (MLB) values, and posterior probabilities (BPP). Voucher numbers and species names are indicated in Table 1.

fungal sequences in the GenBank repository may have erroneous lineage assignations (Bridge et al. 2003; Nilsson et al. 2006). Assessing accuracy in GenBank and other DNA repositories is a key stage for species identification in current biodiversity analyses based on similarity of DNA sequences (Hibbett et al. 2016). It is especially important in cases like *Xylodon paradoxus*, with useful biotechnological properties since, according to Bortolus (2008), a wrong taxonomy could lead not only to inaccurate knowledge of nature, but also to important economic losses.

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Chapter 5

Xylodon jacobaeus sp. nov.

Javier Fernández-López, Margarita Dueñas, María P. Martín, M. Teresa Telleria

ABSTRACT

A new species of *Xylodon* from the Cape Verde archipelago *Xylodon jacobaeus* sp. nov. is described from three specimens at first identified as *Hyphodontia niemelaei* (=*Xylodon niemelaei*) due to their poroid hymenophore and hyohae sistem monomitic, as a part of a broarder study publised in Crous et al (2012) Fungal Planet description sheets: 785–867 Persoonia 41, 238–417. Morphological characteristics along with DNA barcodes are provided as well as comparisons with closely related species.

Xylodon jacobaeus J. Fernández-López, M. Dueñas, M.P. Martín & Telleria, **sp. nov.**, Fig. 1.

Etymology: Named after Santi Jacobi Insula, Latin name for Santiago Island, Cape Verde Archipelago, where it was collected.

Type: CAPE VERDE, Santiago island, São Domingos, Rui Vaz, N15º01'59" W23º37'06", 873 msl, on *Eucalyptus camaldulensis* (Myrtaceae), 21 Sep. 2010, *J. Cardoso, L.M. Catarino, M. Dueñas, M.P. Martín, I. Melo, I. Salcedo & M.T. Telleria*, 18975Tell. (holotype, MA-Fungi 91340, ITS sequence GenBank MH430073, MycoBank MB826918).

Description: Basidioma resupinate, effuse, adnate; hymenophore reticulate to poroid, 1–2 pores/mm, yellowish white to pale yellow (92. y White – 89. p. Y, Kelly & Judd 1976) margin not clearly differentiated, sometimes paler. *Hyphal system* monomitic; hyphae hyaline, thin to slightly thickened walls, sparsely branched, with clamps, 2.5–3.5 µm wide; subicular hyphae loosely interwoven, parallel to substratum; subhymenial hyphae more densely interwoven, perpendicular to substratum, usually slightly encrusted. Cystidia or rather cystidial elements present: 1) capitate cystidia arise from the hymenium, subcylindrical to utriform, thin-walled, basal clamped, 20–24 × 4–7 µm, and 2) capitate hyphae arise from the subiculum, basal clamped, 15–35 × 2.5–3.5 µm, apex up to 7 µm diam. Basidia claviform to subclaviform, sometimes pedunculated, 17–20 × 4–5 µm, internal linear repetition seems to occur occasionally, four sterigmata, with basal clamp. Spores ellipsoid, (5–)6–7 × (3.5–)4–4.5 µm, hyaline, thin-walled, smooth, guttulate, L= 6.24, W=4.35, Q=1.43 (n=32/3) (Fig 2).

Habitat & Distribution: On debris of *Eucalyptus camaldulensis* and *Lantana camara*; known from two localities of Santiago Island, Cape Verde Archipelago (Fig 2).

Additional specimens examined: CAPE VERDE, Santiago island, Santa Catarina, Serra da Malagueta Natural Park, N15º10'41.5" W23º41'14.2", 907 msl, on Lantana camara, 20 Sep. 2010, *J. Cardoso, L.M. Catarino, M. Dueñas, M.P. Martín, I. Melo, I. Salcedo & M.T. Telleria*, 13224MD, MA-Fungi 91338, ITS sequence GenBank MH430074; idem, 13225MD, MA-Fungi 91339, ITS and LSU sequences GenBank MH430072 and MH430071).

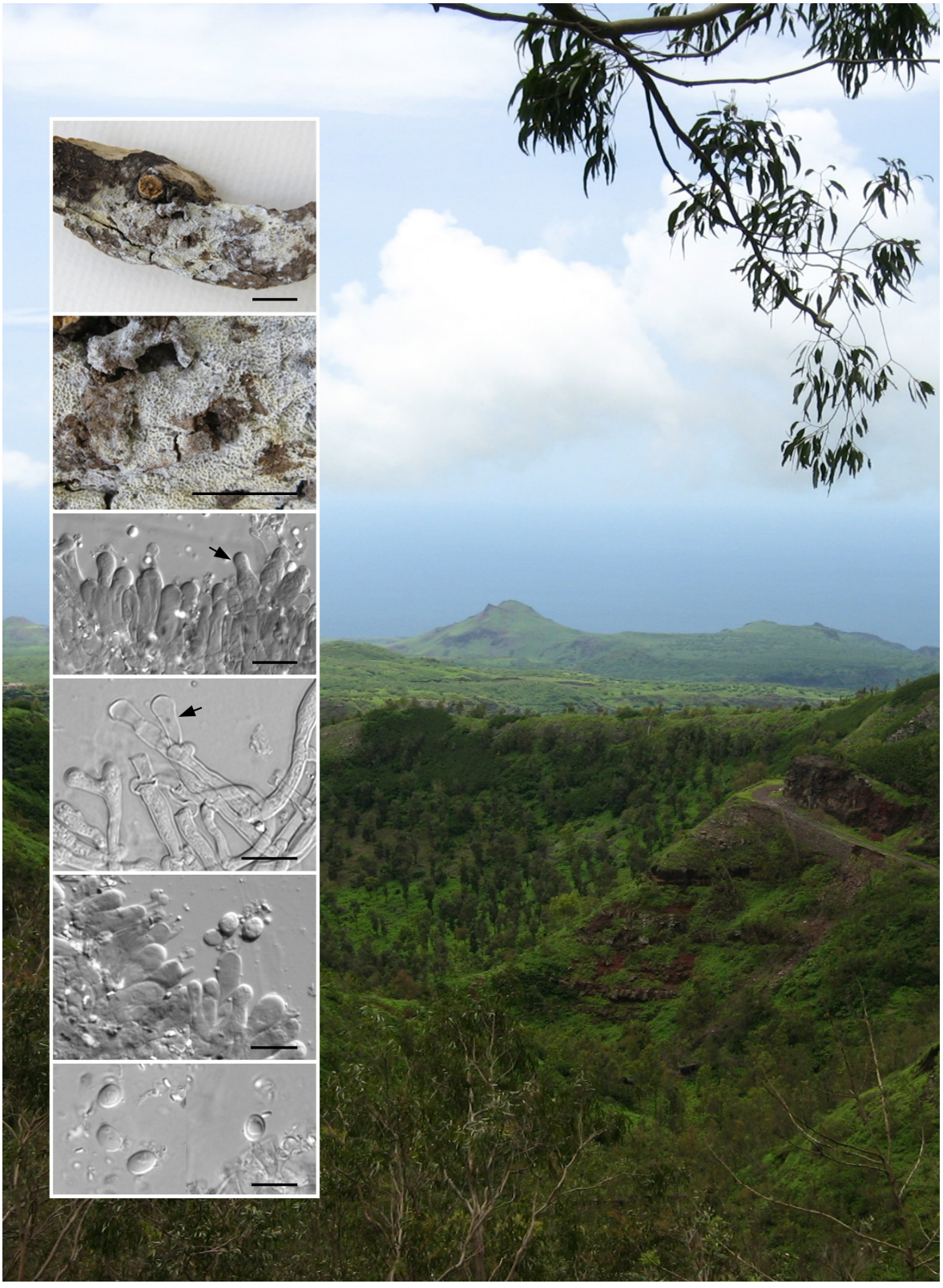

Fig 1. Cape Verde, Santiao, Sao Domingos, Rui Vaz (photo credit M.T. Telleria); from top to bottom: basidioma (MA-Fungi 91340), cystidia, capitate hyphae, basidia and spores (MA-Fungi 91340). Scale bars=1 cm (basidioma), 10 µm (all others).

Notes: Maximum likelihood phylogenetic analyses of ITS sequences under a GTR model grouped the new sequences in a well-supported clade (bootstrap support value > 95 %) with *Xylodon niemelaei*, *X. rhizomorphus*, and *X. reticulatus* (Fig 2). No LSU GenBank sequences were available for *X. reticulatus*. Distribution and morphological diagnostic characters for each species are shown in Table 1. *Xylodon jacobaeus* is similar to these species, but differs in having subcylindrical to utriform cystidia, capitate hyphae and wider spores.

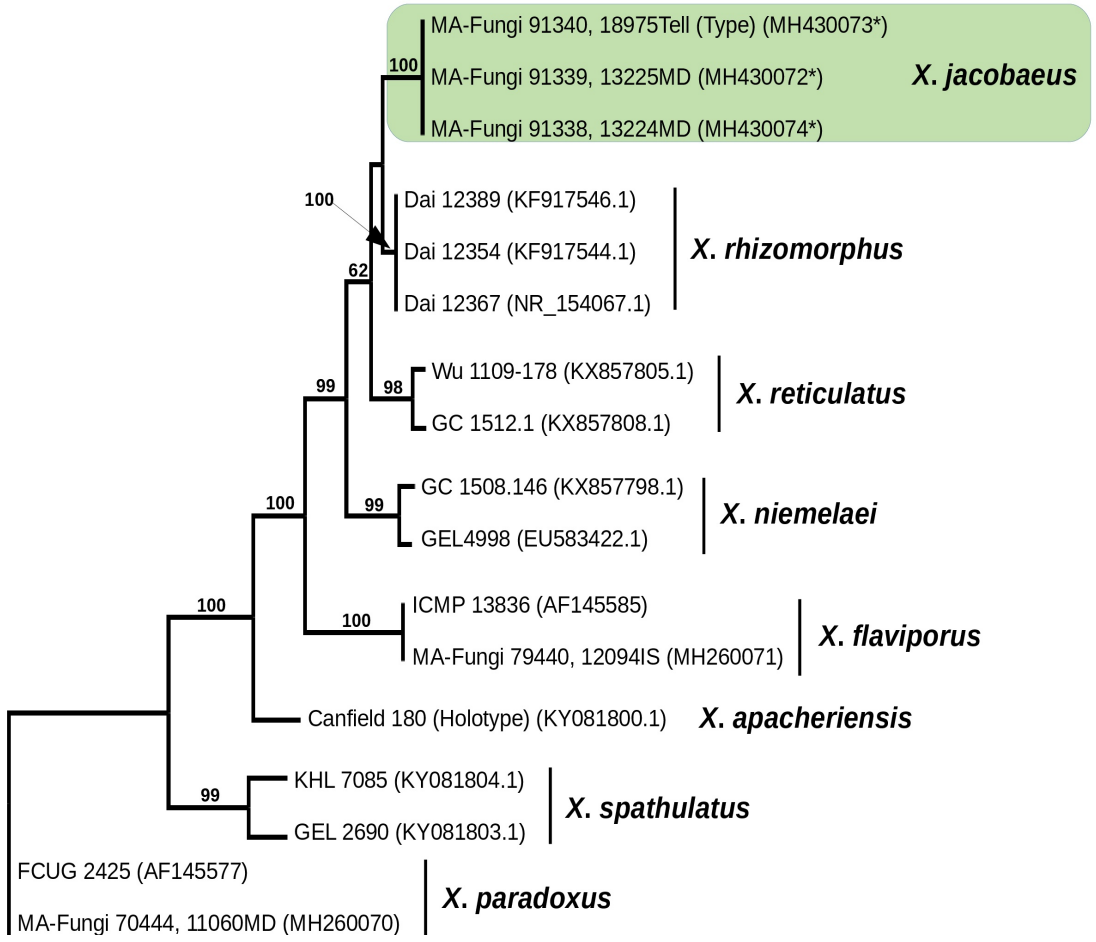

Fig 2. Topology of ITS tree obtained by Maximum Likelihood Inference conducted in RAxML v.8.2.10 on CIPRES Science Gateway v.3.3 (Miller et al. 2010). Two sequences of *X. paradoxus* were used as outgroup. Bootstrap support values (> 50 %) are indicated on the branches (bootstrap iterations=1000). The *X. jacobaeus* clade is marked with a green block; the accession numbers from the EMBL/GenBank database are indicated at the terminal nodes. The asterisk (*) after the EMBL/GenBank accession numbers are sequences obtained for this study.

Table 1. Comparison of distribution and morphology of *Xylodon jacobaeus* and closely related species

Species	Type locality	Cystidia and cystidial elements	Spores	References
X. niemelaei	Taiwan	Capitate and subulate cystidia	5–5.5(–6) × 3.5–4 µm	Wu (1990)
X. rhizomorphus	China	Bladder-like cystidia	(4.1–)4.3–5.5(–5.9) × (3.5–)3.7–4.1(–4.3) µm	Zhao et al. (2014)
X. reticulatus	Taiwan	Capitate, subclavate to clavate and slightly moniliform cystidia; short encrusted hyphal apices	(4.8–)5–6(–7) × 3–3.6(–4) µm	Chen et al. (2017)
X. jacobaeus	Cape Verde	Capitate, subcylindrical to utriform cystidia; capitate hyphae	(5–)6–7 × (3.5–)4–4.5 µm	Present study

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General discussion

Integrative taxonomy approaches addressed in the present thesis dissertation have contributed not only to improve the knowledge about *Xylodon* diversity, but also to better understand the consequences of trait selection to be used for species delimitation in these corticioid fungi. In response to our main working hypothesis, the number and distribution of *Xylodon* species that can be detected depends largely on the characters and methodologies used in their study. In the same context, diagnostic traits that can be used to characterize each species vary among the different approaches.

It has been suggested that *Xylodon* is one of the largest genera of wood-rotting fungi (Hibbet et al. 2014). Riebesehl & Langer (2017) combined into *Xylodon* the species traditionally included in *Schizopora* based on molecular phylogenies. Other recent studies have synonymized with *Xylodon* closely related genera such as *Lagarobasidium* or *Palifer* (Viner et al. 2018; Riebesehl et al. 2019). These studies revealed a high morphological variability within this genus and the importance of molecular data to address the study of *Xylodon* diversity. Our studies are in accordance with these findings, supporting the idea that molecular data are a key trait to delimit *Xylodon* species (Chapters 2–5). The traditional morphological species recognition approach applied to *Xylodon* has been underestimating the true diversity of this genus. In Chapter 1 we showed how this issue could affect the knowledge about *Xylodon* species, such as the geographical distribution inferred for each species, or the environmental conditions associated with them. It is commonly accepted that molecular phylogeny has revolutionized species delimitation and fungal biogeography (Lumbsch et al. 2008). From few and widely distributed species have arisen numerous and geographically restricted species due to the shift from morphological to phylogenetic species recognition approaches (Taylor et al. 2006). This general pattern, explained in Chapter 1 for three *Xylodon* morphospecies, is also known for many basidiomycetes such as *Pleurotus* (Vilgalys & Sun 1994), *Schizophyllum* (James et al. 2001) or the *Hyphoderma setigerum* complex (Nilsson et al. 2003). The split of a single widely distributed species into several more geographically restricted species is confirmed in Chapters 2 and 3. *Xylodon raduloides* is splitted into four closely related but different species using a combination of molecular, morphological and ecological evidence. The geographic distribution of the resulting species is reduced from global to regionally restricted, divided into Europe *Xylodon raduloides*, North America *X. laurentianus*, Patagonia *X. patagonicus* and Australia-New Zealand *X. novozelandicus*. *Xylodon australis* is split into two species reducing their inferred distribution from the Southern

Hemisphere to Australia and New Zealand, while *Xylodon magallanesii* is located in Patagonia.

Results obtained for *Xylodon* are in accordance with the ideas of Taylor et al. (2006) about the geographic structure of fungal species, but not with the hypothesis "*everything is everywhere*" articulated for bacteria by Baas Becking (1934) and generalized for microorganisms and propagules by Finlay (2002).

In contrast to our findings, worldwide phylogenetic species of fungi have been described in some cases. In a study on the ascomycete, *Aspergillus fumigatus*, Pringle et al. (2005) affirmed that "*in all cases in which the phylogenetic species concept has been used to describe species of global isolates of a morphological fungal species, cryptic species have been recognized*". However, although they discovered two phylogenetic species within a global sample of *Aspergillus fumigatus*, no geographic structure was found for these species. In another study of a widespread mould, the basidiomycete *Wallemia sebi*, Nguyen et al. (2015) distinguished four different phylogenetic species using a multi-locus genealogical concordance approach. Two of them were distributed worldwide, with no link between geography and haplotype.

Among *Xylodon* species, *Xylodon flaviporus* has been suggested as an example of a widespread species, from tropical to boreal regions of the world (Langer 1994; Wu 2000). Although no specific phylogenetic studies have been conducted about the distribution range of *Xylodon flaviporus*, Paulus et al. (2000) pointed out its "*surprisingly wide geographical distribution*" in a molecular study conducted on *Schizopora* (=*Xylodon*) species. Some specimens assigned to the morphospecies *Xylodon flaviporus* in Chapter 1 also presented a worldwide distribution following the molecular analyses. These results indicate that *Xylodon flaviporus* could be another case of a worldwide phylogenetic species. However, it is worth stressing the need to confirm this distribution pattern by the addition of new molecular data and other evidence. Even when molecular data are used, wrong inferences often result in a misleading view of fungal diversity, characterized by a lack of endemism and species with a worldwide distribution (Bruns & Taylor 2016). This taxonomic uncertainty has direct consequences in fungal conservation (Mueller & Schmit 2007) or pest risks (Elith et al. 2013) and therefore caution should be taken.

As it has been mentioned above, traditional morphological approaches have generally led to an underestimation of fungal species diversity. Moreover, although the use of multiple DNA markers for species delimitation is increasing, the most recent studies about *Xylodon* diversity have been addressed using only one, ITS (Chen et al. 2018; Shi et

al. 2019) or two loci, ITS and LSU (Riebesehl et al. 2019). Balasundaram et al. (2015) argued that several individual genes performed better than the DNA barcoding marker ITS in resolving phylogenetic species in *Serpula*, recommending the use of a multi-gene analysis for a confident separation of cryptic fungal species. In addition, numerous evolutionary processes generate species tree/gene tree incongruence such as deep coalescence (incomplete lineage sorting), hybridization, etc. (Mallo & Posada 2016), and thus, multi-gene approaches are usually more robust. In the Chapter 2, the inclusion of four DNA markers (ITS, LSU, rpb2 and EF-1alpha) confirmed four different phylogenetic species in the *Xylodon raduloides* complex by a multi-locus species coalescent approach. Moreover, this study supported the effective performance of ITS (Schoch et al. 2014) for species delimitation in *Xylodon*, which allowed us to use this DNA region as the main evidence for species delimitation in Chapters 3 and 5. In addition, although no new species are described in Chapter 4, a multi-gene approach supported the taxonomic identification of the strain KUC8140 as *Xylodon ovisporus*.

This search for evidence in addition to morphological or molecular phylogenetic traits for species delimitation has became a major issue in recent decades (Wiens 2007). In Chapter 2, the study of other sources of evidence such as bioclimatic preferences and spore morphology confirmed that phylogenetic data support environmental and morphological differentiation. Although the use of ecological niche modeling approaches has been argued as a key for species differentiation in many groups: vertebrates (Rissler & Apodaca 2007; Aguilar et al. 2017) or plants, (Frajman et al. 2019), these methodologies are not commonly applied to fungi. In Chapter 2 we showed how these tools can help in the reconstruction of the evolutionary history of *Xylodon* species. Although neither spore morphology nor bioclimatic differences reached the same resolution power as the multi-locus species coalescent approach, the combination of these three different sources of evidence helped us to establish more robust species hypotheses in accordance with the integrative taxonomy framework (Samadi & Barberousse 2006; de Queiroz 2007). Furthermore, the link between environment and morphological traits in fungi has been demonstrated (Kauserud et al. 2008). For this reason, the study of fungal bioclimatic preferences could suggest a search for undetected or hidden morphological variations that would be useful for species recognition.

Integrative taxonomy requires studing species boundaries from multiple and complementary perspectives, but the way those different sources of evidence are combined is not always straightforward. In the Chapters 2 and 5 we use molecular, morphological

and ecological data to describe new *Xylodon* species, but we follow a traditional comparative methodology, checking for concordance between different sources. With the development of molecular phylogeny, morphological character mapping over a phylogenetic tree has been used to understand the evolution and usefulness of morphological traits in order to identify kinship relationships between fungal species (Larsson et al. 2006; Telleria et al. 2010).

At this point, the key role of molecular data in modern taxonomic studies is clear. For this reason, problems arise when the lack of molecular data makes species identification difficult. This issue becomes especially important when it affects type specimens (Ariyawansa et al. 2014), since types fix the application of the species name to an existing entity (Turland et al. 2018). Unfortunately, this situation is very common, espacially when type specimens are too old and it is not easy to obtain good quality DNA to perform phylogenetic analyses. In other cases, those specimens have an intrinsic historical value, and many times destructive sampling is not allowed (Ariyawansa et al. 2014). In Chapter 3 we use a likelihood framework to infer the position of a type specimen from quantitative morphometric data using the *locate.yeti* function from the *phytools* R package (Revell 2012; Revell et al. 2015). Although this method was originally developed to be applied to fossils or recently extinct taxa, the same framework can be used with type specimens for which no molecular data is available. In fungal taxonomic research, the study of the type specimen is always recommended to solve taxonomic issues (Ariyawansa et al. 2014). Therefore, the method used in Chapter 3 and other similar approaches (Parins-Fukuchi 2018) arise as a useful tool to solve future taxonomic questions combining morphological data and molecular phylogeny.

The last step of the integrative taxonomy approach should be to provide a species description, that is, a species name and a diagnosis. Indeed, this is a key step, since it is the only way to ensure that researchers are referring to the same entity. However, it has been proved that the species description is not always provided when a new species is discovered using the integrative taxonomy approach (Pante et al. 2015). In the best scenario, the absence of a species description could result in a loss of value for this research (Goldstein & DeSalle 2011), but worse, it could also cause severe conservation problems (Mace 2004).

Limitations of the study and future perspectives

Integrative taxonomy has been put forward as the best approach to address the study of biodiversity (Padial et al. 2010). Many and independent types of evidence should be combined for a solid and robust study of fungal diversity. In the present thesis dissertation, our studies on *Xylodon* rely mainly on molecular (up to four nDNA regions), morphological, and bioclimatic data, but other sources could be added to complement our findings such as host specificity (Göker et al. 2009), crystal morphology (Larsson et al. 1994), etc.

The number and kind of DNA markers are usually a key decision to study phylogenetic relationships in fungi (Balasundaram et al. 2015). However, the majority of the available studies on fungal diversity are based on LSU or ITS DNA regions, and therefore there is a bias towards these markers in new studies, in order to compare new data with preexisting databases. Recent research has shown the utility of protein-coding regions such as EF-1alpha as a potential secondary barcoding region (Stielow et al. 2015). The shift towards protein-coding regions would allow not only species identification, but also the reconstruction of phylogenetic relationships since these regions are usually more conserved. However, the lack of these markers in public repositories of genetic data is a limiting factor in the use of these DNA regions for taxonomic purposes (Yahr et al. 2016).

In our research, morphological data and environmental preferences were successfully applied for identification of new species in *Xylodon*. However, because these characters could present a high intraspecific variability, large size samples are always recommended in order to perform statistical analysis for trait comparisons between species candidates. In fungal taxonomic studies, a large size sample is usually hard to obtain. Fungi, like insects, is one of the richest group of organisms, but it is still one of the most poorly studied (Purvis & Hector 2000). Since samples for fungal taxonomic research often come from museum or herbarium collections and no additional specimens are available to study, sample size is usually limited. Further studies would increase sample size for the species studied in the present thesis dissertation, and more robust analyses could be addressed.

Xylodon diversity is still far from being entirely known. Despite the recent studies focusing on this genus (Riebesehl & Langer 2017; Fernández-López et al. 2018, 2019; Riebesehl et al. 2019; Shi et al. 2019), its great diversity and morphological variability requires more effort to achieve a better knowledge of their species and the evolutionary

processes generating them. Hawksworth & Rossman (1997) estimated that around 20,000 new fungal species waiting to be described could be already collected and stored in herbaria, although this number is probably underestimated. In this context, museums and herbaria collections will play a major role in preserving and providing samples to study *Xylodon* and the rest of fungal diversity.

CONCLUSIONS

1- Species recognition criteria used in the study of *Xylodon* diversity significantly affect the species delimitation, and therefore the number of species that can be distinguished, as well as the distribution patterns inferred for each species. Characters such as bioclimatic preferences can be helpful traits to be used as supplementary evidence to distinguish among taxa in *Xylodon*.

2- The multi-locus multi-species coalescent approach is useful to contrast among different hypotheses of species arrangements in *Xylodon*, as well as to discover the hidden diversity under a single species name. In addition, the inclusion of multiple and complementary sources of evidence (morphological, ecological, molecular, etc.) in an integrative taxonomy framework helps to delimit *Xylodon* species boundaries.

3- Quantitative morphological characters can be used to locate a sample within a molecular phylogeny using a maximum likelihood framework through the *locate.yeti* function from the *phytools* R package. This can help to solve taxonomic issues when used to assign a type specimen to a specific clade.

4- Despite the recent interest in *Xylodon*, its diversity is still unknown and further studies are required to understand the evolutionary processes that generate this diversity and distribution. Poorly explored regions are a source of undiscovered fungal species, but also collections in museums and herbaria play a major role in preserving and providing samples to study *Xylodon*.

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Appendix

List of other publications and manuscripts coauthored by Javier Fernández-López during the development of the PhD thesis

Martín MP, Zhang LF, **Fernández-López J**, Dueñas M, RodríguezArmas JL, Beltrán-Tejera E, Telleria MT (2018) *Hyphoderma paramacaronesicum* sp. nov. (Meruliaceae, Polyporales, Basidiomycota), a cryptic lineage to *H. macaronesicum*. Fungal Systematics and Evolution 2(1): 5768. https://doi.org/10.3114/fuse.2018.02.05

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Telleria MT, Dueñas M, Melo I, Salcedo I, Cardoso J, **Fernández-López J,** Martín MP (2016) Corticioid fungi (Basidiomycota) from the Biosphere Reserve of Arganeraie, Morocco: a preliminary survey. Nova Hedwigia, 103(1-2): 193–210. https://doi.org/10.1127/nova_hedwigia/2016/0345

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Aunque todo viaje comienza con un primer paso, a veces resulta difícil identificar si fue esta o aquella decisión la que inició el camino. Lo que sí resulta más sencillo es acordarte de aquellas personas que te han acompañado durante el trayecto. Como en cualquier viaje, sobre todo si se hace largo y duro, esas personas son tantas que resultaría imposible y casi absurdo individualizarlas a todas. El personal de la Complutense con el que comencé el camino, las compañeras y compañeros del RJB con los que compartí mis días durante 5 años, o la inesperada familia del IREC que tan bien me ha acogido. Las personas de fuera del ámbito científico, que nos ayudan a recordar que hay otro mundo más allá de los *papers*, factores de impacto o el éxito de las carreras investigadoras (que tanta falta hace a veces). Todas ellas también forman parte de este trabajo, y cerrar esta etapa no habría sido posible sin su ayuda.

La familia suele constituir un pilar fundamental en el que apoyarse en los momentos más difíciles. En parte, por esa extraña incondicionalidad que a menudo se da entre los familiares a pesar de las diferencias, y que muchas veces se hace tan necesaria para superar las malas rachas. Mi caso no ha sido una excepción. Agradezco a toda mi familia y en especial a mi madre, mi padre y mi hermana por su apoyo en cada una de las pruebas que juntos hemos ido superando. Sin ellos habría sido mucho más duro.

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